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(54) Title: POLYNUCLEOTIDE AND ITS USE FOR MODULATING A DEFENCE RESPONSE IN PLANTS

## (57) Abstract

The cloning of the *Mlo* gene of Barley, *mlo* mutants, and homologues from other species including rice and *Arabidopsis thaliana* enables manipulation of pathogen defence responses in plants. Nucleic acid and polypeptides are used in the production of transgenic plants in which the pathogen defence response is modulated, particularly stimulated. Various approaches enable diagnostic determination of the presence of susceptibility or resistance alleles in plants. Compounds able to modulate a defence response by interaction with Mlo or mlo protein are identifiable using assays involving the protein or fragments thereof.

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## POLYNUCLEOTIDE AND ITS USE FOR MODULATING A DEFENCE RESPONSE IN PLANTS

The present invention relates to stimulating a defence response in plants, with a view to providing the plants with enhanced pathogen resistance. More specifically, it has resulted from cloning of the barley *Mlo* gene, various mutant *mlo* alleles, and a number of homologues from various species. The *Mlo* gene has been isolated using a positional cloning approach which has never previously been successful in Barley. Details and discussion are provided below. Wild-type *Mlo* exerts a negative regulatory function on a pathogen defence response, such that mutants exhibit a defence response in the absence of pathogen. In accordance with the present invention, down-regulation or out-competition of *Mlo* function may be used to stimulate a defence response in transgenic plants, conferring increased pathogen resistance.

Mutations have been described in several plants in which defence responses to pathogens appear to be constitutively expressed. Mutation-induced recessive alleles (*mlo*) of the barley *Mlo* locus exhibit a leaf lesion phenotype and confer an apparently durable, broad spectrum resistance to the powdery mildew pathogen, *Erysiphe graminis* f sp *hordei*.

Resistance responses to the powdery mildew pathogen have been genetically well characterized (Wiberg, 1974; Søgaard and Jørgensen, 1988; Jørgensen, 1994). In most analyzed cases resistance is specified by race-specific resistance genes following the rules of Flor's gene-for-gene hypothesis (Flor, 1971). In this type of plant/pathogen interaction, resistance

is specified by and dependent on the presence of two complementary genes, one from the host and one from the fungal pathogen. The complementary genes have been termed operationally (pathogen) resistance ("R") gene and avirulence gene, respectively. Most of the powdery mildew resistance genes (*Mlx*) act as dominant or semidominant traits (Jørgensen, 1994).

Monogenic resistance mediated by recessive (*mlo*) alleles of the *Mlo* locus is different. Apart from being recessive, it differs from race-specific resistance to single pathogen strains in that (i) it confers broad spectrum resistance to almost all known isolates of the pathogen (ii) *mlo* resistance alleles have been obtained by mutagen treatment of any tested susceptible wild type (*Mlo*) variety, and (iii) *mlo* resistance alleles exhibit a defence mimic phenotype in the absence of the pathogen (Wolter et al. , 1993). Thus, the genetic data indicate the *Mlo* wild type allele exerts a negative regulatory function on defence responses to pathogen attack.

Resistance mediated by *mlo* alleles is currently widely used in barley breeding and an estimated 10 million hectares are annually planted in Europe with seeds of this genotype. A 'mlo like' inherited resistance to powdery mildew in other cereal plants has not been reported so far although the fungus is a relevant pathogen in wheat (attacked by *Erysiphe graminis* f sp *tritici*), oat (attacked by *E. g.* f sp *avenae*), and rye (attacked by *E. g.* f sp *secalis*). Because cereals are morphologically, genetically and biochemically highly related to each other (Moore et al., 1995), one would predict the



homologous genes in these species. The failure to  
'mlo like' inherited resistance in wheat and oat  
due to their hexaploid genomes, making it difficult  
mutagenesis defective alleles in all six gene  
the chance of all such mutations occurring in  
the. The failure to have found a *mlo* equivalent  
this is probably due to insignificant amount of  
analysis in these species and complications as a  
outbreeding nature (e.g. rye).

genes closely linked to *Mlo* on barley chromosome 4  
identified on the basis of a *mlo* backcross line  
involving *mlo* alleles from six genetic backgrounds  
(1991). The map position of *Mlo* on the basis of  
is consistent with its chromosomal localization  
from a previous mapping with morphological markers

defined an ~3cM genetic interval containing *Mlo*  
markers, we decided to attempt to isolate  
by DNA cloning.

There is no documented example of a successful  
attempt of a barley gene. We were faced  
with difficulties.

Size of barley ( $5.3 \times 10^9$  bp/haploid genome  
and Smith, 1991) has almost double the size  
and because the total genetic map covers  
(1995) we were confronted with a very  
large genetic and physical distances (1 cM

corresponds to ~ 3 Mb).

Secondly, a high resolution genetic map had to be constructed around *Mlo* enabling the positioning of linked markers with a precision of better than 0.1 cM.

5       Thirdly, we aimed to physically delimit the target gene and both flanking DNA markers on individual large insert genomic clones, a procedure later termed "chromosome landing" (Tanksley et al., 1995). For this purpose, a complete barley YAC library from barley Megabase DNA had to be constructed with  
10   an average insert size of 500-600 kb, which was unprecedented.

Fourthly, we had to prepare unusual genetic tools that enabled us to identify the *Mlo* gene within a physically delimited region without the need for a time consuming generation of barley transgenic plants and testing of different candidate genes. We used for our studies ten characterized radiation- or chemically-induced *mlo* mutants (Jørgensen, 1992). For a conclusive chain of evidence of the gene isolation we decided to depend upon a functional restoration of the wild type *Mlo* allele starting out from characterized *mlo* defective alleles. For this purpose, we performed *mlo* heteroallelic crosses and isolated susceptible intragenic *Mlo* recombinants. The sequence analysis of these proves the function of the described gene.

The cloning of the barley *Mlo* gene and homologues, including homologues from other plant species, gives rise to a number of practical applications, reflected in the various aspects of the present invention.

According to a first aspect of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence encoding a peptide with Mlo function. Those skilled in the art will appreciate that "Mlo function" refers to the ability to suppress a defence response, said defence response being race and/or pathogen independent and autonomous of the presence of a pathogen, such as, for example, the *Mlo* gene of barley, the *Acd* gene and the *Lsd* gene of *Arabidopsis*.

*mlo* mutations that down-regulate or disrupt functional expression of the wild-type Mlo sequence are recessive, such that they are complemented by expression of a wild-type sequence. Thus "Mlo function" can be determined by assessing the level of constitutive defence response and/or susceptibility of the plant to a pathogen such as, for example, powdery mildew or rust (e.g. yellow rust). Accordingly, a putative nucleotide sequence with *Mlo* function can be tested upon complementation of a suitable *mlo* mutant. The term "mlo function" is used to refer to sequences which confer a *mlo* mutant phenotype on a plant.

The capitalisation of "Mlo" and non-capitalisation of "mlo" is thus used to differentiate between "wild-type" and "mutant" function.

A *mlo* mutant phenotype is characterised by the exhibition of an increased resistance against one or more pathogens, which is race and/or pathogen independent and autonomous of the presence of a pathogen.

The test plant may be monocotyledonous or dicotyledonous.

Suitable monocots include any of barley, rice, wheat, maize or oat, particularly barley. Suitable dicots include *Arabidopsis*.

Nucleic acid according to the invention may encode a  
5 polypeptide comprising the amino acid sequence shown in Figure 2, or an allele, variant, derivative or mutant, or homologue, thereof.

Nucleic acid according to the present invention may have the sequence of a *Mlo* gene of barley, or be a mutant, variant  
10 (or derivative) or allele of the sequence provided, or a homologue thereof. Preferred mutants, variants and alleles are those which encode a sequence which retains a functional characteristic of the wild-type gene, especially the ability to suppress a defence response as discussed herein. Other  
15 preferred mutants, variants and alleles encode a sequence which, in a homozygote, cause constitutive activation of a defence response, or at least promotes activation of a defence response (i.e. is a *mlo* mutant sequence), e.g. by reducing or wholly or partly abolishing *Mlo* function. Preferred mutations  
20 giving *mlo* mutant sequences are shown in Table 1. Changes to a sequence, to produce a mutant, derivative or variant, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion and/or substitution of one or  
25 more amino acids. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence are included. Particular variants, mutants, alleles and

derivatives are discussed further below, as well as homologues.

A preferred nucleic acid sequence according to an aspect of the present invention is shown in Figure 2 along with the predicted amino acid sequence. Nucleic acid may be subject to  
5 alteration by way of substitution of nucleotides and/or a combination of addition, insertion and/or substitution of one or more nucleotides with or without altering the encoded amino acids sequence (by virtue of the degeneracy of the genetic code).

10 As discussed below, further aspects of the present invention provide homologues of the *Mlo* sequence shown in Figure 2, including from rice (genomic sequence Figure 5, bottom line, cDNA sequence Figure 10, amino acid sequence Figure 13) and barley (genomic sequence Figure 6, bottom line,  
15 cDNA sequence Figure 11, amino acid sequence Figure 14); also Table 5B (nucleotide sequences) and Figure 5A (amino acid sequences) show homologous EST's from rice and *Arabidopsis*.

The present invention also provides a vector which comprises nucleic acid with any one of the provided sequences,  
20 preferably a vector from which a product can be expressed. The vector is preferably suitable for transformation into a plant cell and/or a microbial cell. The invention further encompasses a host cell transformed with such a vector, especially a plant cell or a microbial cell (e.g. *Agrobacterium tumefaciens*).

25 Thus, a host cell, such as a plant cell, comprising nucleic acid according to the present invention is provided. Within the cell, the nucleic acid may be incorporated within the nuclear

genome, i.e. a chromosome. There may be more than one heterologous nucleotide sequence per haploid genome.

A vector comprising nucleic acid according to the present invention need not include a promoter, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

Nucleic acid molecules and vectors according to the present invention may be provided in a form isolated and/or purified from their natural environment, in substantially pure or homogeneous form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the relevant sequence. Nucleic acid according to the present invention may comprise cDNA, RNA, genomic DNA and may be wholly or partially synthetic. The term "isolate" may encompass all these possibilities.

The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and methods of making the expression product by expression from encoding nucleic acid therefore under suitable conditions in suitable host cells, e.g. *E. coli*. Those skilled in the art are well able to construct vectors and design protocols for expression and recovery of products of recombinant gene expression. Suitable vectors can be chosen or constructed, containing one or more appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for

example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Transformation procedures depend on the host used, but are well known. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992.

1 The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference, along with all other documents mentioned.

Purified Mlo protein, or a fragment, mutant or variant thereof, e.g. produced recombinantly by expression from  
5 encoding nucleic acid therefor, may be used to raise antibodies employing techniques which are standard in the art. Antibodies and polypeptides comprising antigen-binding fragments of antibodies may be used in identifying homologues from other species as discussed further below.

0 Methods of producing antibodies include immunising a mammal (eg human, mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and might be screened, preferably  
5 using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, *Nature* 357: 80-82).

Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal, antibodies with appropriate binding specificity may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, eg using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibodies raised to a polypeptide or peptide can be used in the identification and/or isolation of homologous polypeptides, and then the encoding genes. Thus, the present invention provides a method of identifying or isolating a polypeptide with Mlo or mlo function (in accordance with embodiments disclosed herein), comprising screening candidate peptides or polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind an Mlo or mlo peptide, polypeptide or fragment, variant or variant thereof or preferably has binding specificity for such a peptide or polypeptide, such as having an amino acid sequence identified herein. Specific binding members such as antibodies and polypeptides comprising antigen binding domains of antibodies that bind and are preferably specific for a Mlo or mlo peptide or polypeptide or mutant, variant or derivative hereof represent further aspects of the present invention, as to their use and methods which employ them.

Candidate peptides or polypeptides for screening may for



instance be the products of an expression library created using nucleic acid derived from an plant of interest, or may be the product of a purification process from a natural source.

A peptide or polypeptide found to bind the antibody may be isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the peptide or polypeptide either wholly or partially (for instance a fragment of a polypeptide may be sequenced). Amino acid sequence information may be used in obtaining nucleic acid encoding the peptide or polypeptide, for instance by designing one or more oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridisation to candidate nucleic acid, or by searching computer sequence databases, as discussed further below.

A further aspect of the present invention provides a method of identifying and cloning *Mlo* homologues from plants, including species other than Barley, which method employs a nucleotide sequence derived from that shown in Figure 2. Further similar aspects employ a nucleotide sequence derived from any of the other Figures provided herein. Nucleic acid libraries may be screened using techniques well known to those skilled in the art and homologous sequences thereby identified then tested. The provision of sequence information for the *Mlo* gene of Barley and various homologues enables the obtention of homologous sequences from Barley and other plant species, as exemplified further herein.

Also, one can easily derive PCR primers based on putative

exon sequences, which might be identified by comparison with the *Mlo* sequence provided in Figure 2 wherein exons are highlighted, and perform RT-PCR with total RNA from the plant of interest, e.g. barley and rice for the homologues shown in  
5 Figures 5 and 6, with cDNA and amino acid sequences shown in other figures herein.

The homologues whose nucleotide sequences are given and whose amino acid sequences are given or are deducible represent and provide further aspects of the present invention in  
10 accordance with those disclosed for the Barley gene shown in Figure 2.

The present invention also extends to nucleic acid encoding a *Mlo* homologue obtained using a nucleotide sequence derived from that shown in Figure 2, or the amino acid sequence  
5 shown in Figure 2. Preferably, the nucleotide sequence and/or amino acid sequence shares homology with the sequence encoded by the nucleotide sequence of Figure 2, preferably at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80% homology, or at least about 85% homology, or at least about 90% homology, most preferably at least about 95% homology. "Homology" in relation to an amino acid sequence may be used to refer to identity or similarity, preferably identity. High levels of amino acid identity may be limited to functionally significant domains or regions.

A mutant, allele, variant or derivative amino acid sequence in accordance with the present invention may include

within the sequence shown in Figure 2, a single amino acid change with respect to the sequence shown in Figure 2, or 2, 3, 4, 5, 6, 7, 8, or 9 changes, about 10, 15, 20, 30, 40 or 50 changes, or greater than about 50, 60, 70, 80 or 90 changes.

5 In addition to one or more changes within the amino acid sequence shown in Figure 2, a mutant, allele, variant or derivative amino acid sequence may include additional amino acids at the C-terminus and/or N-terminus.

As is well-understood, homology at the amino acid level is  
10 generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine,  
15 glutamic for aspartic acid, or glutamine for asparagine. Similarity may be as defined and determined by the TBLASTN program, of Altschul et al. (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art, or, and this may be preferred, the standard program BestFit, which is part of the  
20 Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). BestFit makes an optimal alignment of the best segment of similarity between two sequences. Optimal alignments are found by inserting gaps to maximize the number  
25 of matches using the local homology algorithm of Smith and Waterman

Homology may be over the full-length of the relevant

sequence shown herein, or may more preferably be over a contiguous sequence of about or greater than about 20, 25, 30, 33, 40, 50, 67, 133, 167, 200, 233, 267, 300, 333, 400, 450, 500, 550, 600 or more amino acids or codons, compared with the relevant amino acid sequence or nucleotide sequence as the case may be.

The EST sequences provided herein, have on average 70% similarity and 50% identity with the Mlo amino acid sequence of Figure 2. We show that the rice homologue (Figure 5) and barley homologue (Figure 6) have an amino acid identity of 81% (amino acid sequences shown in Figure 13 and Figure 14).

In certain embodiments, an allele, variant, derivative, mutant or homologue of the specific sequence may show little overall homology, say about 20%, or about 25%, or about 30%, or about 35%, or about 40% or about 45%, with the specific sequence. However, in functionally significant domains or regions the amino acid homology may be much higher. Putative functionally significant domains or regions can be identified using processes of bioinformatics, including comparison of the sequences of homologues. Functionally significant domains or regions of different polypeptides may be combined for expression from encoding nucleic acid as a fusion protein. For example, particularly advantageous or desirable properties of different homologues may be combined in a hybrid protein, such that the resultant expression product, with Mlo or mlo function, may comprise fragments of various parent proteins.

The nucleotide sequence information provided herein, or

any part thereof, may be used in a data-base search to find homologous sequences, expression products of which can be tested for *Mlo* or *mlo* function. These may have ability to complement a *mlo* mutant phenotype in a plant or may, upon  
5 expression in a plant, confer a *mlo* phenotype.

In public sequence databases we recently identified several homologues for the sequence of Figure 2. We have already found homologues in rice and barley, and the dicot. *Arabidopsis*.

10 By sequencing homologues, studying their expression patterns and examining the effect of altering their expression, genes carrying out a similar function to *Mlo* in Barley are obtainable. Of course, mutants, variants and alleles of these sequences are included within the scope of the present  
15 invention in the same terms as discussed above for the Barley gene.

Homology between the homologues as disclosed herein, may be exploited in the identification of further homologues, for example using oligonucleotides (e.g. a degenerate pool)  
20 designed on the basis of sequence conservation.

According to a further aspect, the present invention provides a method of identifying or a method of cloning a *Mlo* homologue, e.g. from a species other than Barley, the method employing a nucleotide sequence derived from that shown in  
25 Figure 2 or that shown in any of the other Figures herein. For instance, such a method may employ an oligonucleotide or oligonucleotides which comprises or comprise a sequence or

sequences that are conserved between the sequences of Figures 2 and/or 5 and/or 6 and/or 10 and/or 11 and/or 12, or encoding an amino acid sequence conserved between Figure 2 and/or 7 and/or 13 and/or 14 and/or 15 to search for homologues. Thus, a  
5 method of obtaining nucleic acid is provided, comprising hybridisation of an oligonucleotide or a nucleic acid molecule comprising such an oligonucleotide to target/candidate nucleic acid. Target or candidate nucleic acid may, for example, comprise a genomic or cDNA library obtainable from an organism known to contain or suspected of containing such nucleic acid, either monocotyledonous or dicotyledonous. Successful hybridisation may be identified and target/candidate nucleic acid isolated for further investigation and/or use.

Hybridisation may involve probing nucleic acid and identifying positive hybridisation under suitably stringent conditions (in accordance with known techniques) and/or use of oligonucleotides as primers in a method of nucleic acid amplification, such as PCR. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

As an alternative to probing, though still employing nucleic acid hybridisation, oligonucleotides designed to amplify DNA sequences may be used in PCR reactions or other methods involving amplification of nucleic acid, using routine

for instance "PCR protocols; A Guide to  
 rations", Eds. Innis et al, 1990, Academic

no acid sequences suitable for use in the  
 r PCR primers for some purposes are sequences  
 ely, substantially or partly) between at  
 des or polypeptides encoded by genes able to  
 response in a plant, e.g. with any of the  
 s of any of the various figures herein  
 re nucleotide sequences of any of the  
 in.

amino acid sequence information  
 es or primers may be designed, taking into  
 y of the genetic code, and, where  
 age of the organism from the candidate  
 ed.

gonucleotide in accordance with certain  
 ention, e.g. for use in nucleic acid  
 o about 50 nucleotides, or about 40  
 ) or fewer nucleotides in length (e.g.

her or not such a PCR product  
 logue genes may be conducted in various  
 ich a reaction might contain a complex  
 dual products may be cloned and each  
 d. It may be analysed by  
 function on introduction into a plant

rest.

noted, nucleic acid according to the present invention  
able using oligonucleotides, designed on the basis of  
information provided herein, as probes or primers.  
id isolated and/or purified from one or more cells of  
another plant (see above), or a nucleic acid library  
om nucleic acid isolated and/or purified from the  
a cDNA library derived from mRNA isolated from the  
be probed under conditions for selective  
n and/or subjected to a specific nucleic acid  
a reaction such as the polymerase chain reaction  
ucleic acid probed or used as template in the  
reaction may be genomic DNA, cDNA or RNA. If  
or more gene fragments may be ligated to  
l-length coding sequence.

tested several PCR primers derived from the *Mlo*  
sed herein to test their specificity for  
ic acid according to the present invention,  
y genomic DNA and RT-PCR templates. The latter  
from barley polyA<sup>+</sup> RNA. In each case we were  
he expected *Mlo* derived gene fragments as  
ind subsequent DNA sequencing of the PCR  
ngth cDNA clones can be obtained as described  
technology if RT-PCR products are used as

imers tested include:



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25L        5'-GTG CAT CTG CGT GTG CGT A-3'  
25LN       5'-GTG TGC GTA CCT GGT AGA G-3'  
25R        5'-AAC GAC GTC TGG TGC GTG-3'  
33         5'-TGC AGC TAT ATG ACC TTC CCC CTC-3'  
37         5'-GGA CAT GCT GAT GGC TCA GA-3'  
38         5'-CAG AAC TTG TCT CAT CCC TG-3  
38A        5'-GGC TAT ACA TTG GGA CTA ACA-3'  
38B        5'-CGA ATC ATC ACA TCC TAT GTT-3'  
39         5'-GCA AGT TCG ACT TCC AC-3'  
39A        5'-TCG ACT TCC ACA AGT ACA TCA-3'  
          5'-AGC GTA CCT GCG TAC GTA G-3'

rious primer combinations have been tested:

38/39; 38/33; 38/37; 38A/39A; 38B/39A; 38/25L; 38/25LN;  
25R/25LN; 25R/53.

ious aspects of the present invention include the  
le nucleic acid, methods of screening material, e.g.  
ate, nucleic acid preparations, for the presence of  
acid of interest, methods of obtaining the nucleic  
the primers and primer combinations given above.

sequence information provided herein also allows the  
diagnostic tests for determination of the presence of  
mlo resistance allele, or a susceptibility allele  
(-type), in any given plant, cultivar, variety,  
landrace, part of a family or other selection in a  
programme or other such genotype. A diagnostic test

may be based on determination of the presence or absence of a particular allele by means of nucleic acid or polypeptide determination.

At the nucleic acid level, this may involve hybridisation of a suitable oligo- or poly-nucleotide, such as a fragment of the *Mlo* gene or a homologue thereof, including any homologue disclosed herein, or any particular allele, such as an allele which gives an *mlo* phenotype, such as any such allele disclosed herein. The hybridisation may involve PCR designed to amplify a product from a given allelic version of *mlo*, with subsequent detection of an amplified product by any of a number of possible methods including but not limited to gel electrophoresis, capillary electrophoresis, direct hybridisation of nucleotide sequence probes and so on. A diagnostic test may be based on PCR designed to amplify various alleles or any allele from the *Mlo* locus, with a test to distinguish the different possible alleles by any of a number of possible methods, including DNA fragment size, restriction site variation (e.g. CAPS - cleaved amplified polymorphic sites) and so on. A diagnostic test may also be based on a great number of possible variants of nucleic acid analysis that will be apparent to those skilled in the art, such as use of a synthetic *mlo*-derived sequence as a hybridisation probe.

Broadly, the methods divide into those screening for the presence of nucleic acid sequences and those that rely on detecting the presence or absence of a polypeptide. The methods may make use of biological samples from one or more

plants or cells that are suspected to contain the nucleic acid sequences or polypeptide.

Exemplary approaches for detecting nucleic acid or polypeptides include analysing a sample from the plant or plant  
5 cell by:

(a) comparing the sequence of nucleic acid in the sample with all or part of the nucleotide sequence shown in Figure 7 to determine whether the sample from the patient contains a mutation;

10 (b) determining the presence in the sample of a polypeptide including the amino acid sequence shown in Figure 2 or a fragment thereof and, if present, determining whether the polypeptide is full length, and/or is mutated, and/or is expressed at the normal level;

15 (c) performing DNA fingerprinting to compare the restriction pattern produced when a restriction enzyme cuts nucleic acid in the sample with the restriction pattern obtained from the nucleotide sequence shown in Figure 7 or from a known mutant, allele or variant thereof;

20 (d) contacting the sample with a specific binding member capable of binding to nucleic acid including the nucleotide sequence as set out in Figure 7 or a fragment thereof, or a mutant, allele or variant thereof, the specific binding member including nucleic acid hybridisable with the sequence of Figure  
25 7 or a polypeptide including a binding domain with specificity for nucleic acid including the sequence of Figure 7 or the polypeptide encoded by it, or a mutated form thereof, and

determining binding of the specific binding member;

(e) performing PCR involving one or more primers based on the nucleotide sequence shown in Figure 7 to screen the sample for nucleic acid including the nucleotide sequence of Figure 7  
5 or a mutant, allele or variant thereof.

When screening for a resistance allele nucleic acid, the nucleic acid in the sample will initially be amplified, e.g. using PCR, to increase the amount of the analyte as compared to other sequences present in the sample. This allows the target  
10 sequences to be detected with a high degree of sensitivity if they are present in the sample. This initial step may be avoided by using highly sensitive array techniques that are becoming increasingly important in the art.

A variant form of the gene may contain one or more  
15 insertions, deletions, substitutions and/or additions of one or more nucleotides compared with the wild-type sequence (such as shown in Table 1) which may or may not disrupt the gene function. Differences at the nucleic acid level are not necessarily reflected by a difference in the amino acid  
20 sequence of the encoded polypeptide. However, a mutation or other difference in a gene may result in a frame-shift or stop codon, which could seriously affect the nature of the polypeptide produced (if any), or a point mutation or gross mutational change to the encoded polypeptide, including  
5 insertion, deletion, substitution and/or addition of one or more amino acids or regions in the polypeptide. A mutation in a promoter sequence or other regulatory region may prevent or

reduce expression from the gene or affect the processing or stability of the mRNA transcript.

Tests may be carried out on preparations containing genomic DNA, cDNA and/or mRNA. Testing cDNA or mRNA has the advantage of the complexity of the nucleic acid being reduced by the absence of intron sequences, but the possible disadvantage of extra time and effort being required in making the preparations. RNA is more difficult to manipulate than DNA because of the wide-spread occurrence of RN'ases.

Nucleic acid in a test sample may be sequenced and the sequence compared with the sequence shown in Figure 2, or other figure herein, to determine whether or not a difference is present. If so, the difference can be compared with known susceptibility alleles (e.g. as summarised in Table 1) to determine whether the test nucleic acid contains one or more of the variations indicated, or the difference can be investigated for association with disease resistance.

The amplified nucleic acid may then be sequenced as above, and/or tested in any other way to determine the presence or absence of a particular feature. Nucleic acid for testing may be prepared from nucleic acid removed from cells or in a library using a variety of other techniques such as restriction enzyme digest and electrophoresis.

Nucleic acid may be screened using a variant- or allele-specific probe. Such a probe corresponds in sequence to a region of the gene, or its complement, containing a sequence alteration known to be associated with disease resistance.

Under suitably stringent conditions, specific hybridisation of such a probe to test nucleic acid is indicative of the presence of the sequence alteration in the test nucleic acid. For efficient screening purposes, more than one probe may be used  
5 on the same test sample.

Allele- or variant-specific oligonucleotides may similarly be used in PCR to specifically amplify particular sequences if present in a test sample. Assessment of whether a PCR band contains a gene variant may be carried out in a number of ways  
10 familiar to those skilled in the art. The PCR product may for instance be treated in a way that enables one to display the mutation or polymorphism on a denaturing polyacrylamide DNA sequencing gel, with specific bands that are linked to the gene variants being selected.

15 An alternative or supplement to looking for the presence of variant sequences in a test sample is to look for the presence of the normal sequence, e.g. using a suitably specific oligonucleotide probe or primer.

Approaches which rely on hybridisation between a probe and  
20 test nucleic acid and subsequent detection of a mismatch may be employed. Under appropriate conditions (temperature, pH etc.), an oligonucleotide probe will hybridise with a sequence which is not entirely complementary. The degree of base-pairing between the two molecules will be sufficient for them to anneal  
5 despite a mis-match. Various approaches are well known in the art for detecting the presence of a mis-match between two annealing nucleic acid molecules.

RNase A cleaves at the site of a mis-match.ected by electrophoresing test nucleic acidnt probe or probe has annealed and lookingas (i.e. molecules with higherlity) than the full length probe/testaches rely on the use of enzymes such as leases.

cleotide probe that has the sequence of a gene (either sense or anti-sense strand)ociated with disease resistance are knownle 1) may be annealed to test nucleicr absence of a mis-match determined.ce of a mis-match may indicate theleic acid of a mutation associated withthe other hand, an oligonucleotideace of a region of the gene including a disease resistance may be annealed to presence or absence of a mis-matchof a mis-match may indicate that the ample has the normal sequence, or a sequence. In either case, a batteryons of the gene may be employed.ences in sequence of nucleic acidmeans of restriction enzymeod of DNA fingerprinting where thewhen one or more restrictionple of nucleic acid is compared

with the pattern obtained when a sample containing the normal gene or a variant or allele is digested with the same enzyme or enzymes.

The presence or absence of a lesion in a promoter or other regulatory sequence may also be assessed by determining the  
5 level of mRNA production by transcription or the level of polypeptide production by translation from the mRNA.

Nucleic acid isolated and/or purified from one or more cells of a plant or a nucleic acid library derived from nucleic  
10 acid isolated and/or purified from cells (e.g. a cDNA library derived from mRNA isolated from the cells), may be probed under conditions for selective hybridisation and/or subjected to a specific nucleic acid amplification reaction such as the polymerase chain reaction (PCR).

A method may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridisation events and isolate hybridised nucleic acid.

Binding of a probe to target nucleic acid (e.g. DNA) may be measured using any of a variety of techniques at the



those skilled in the art. For instance, probes may  
ely, fluorescently or enzymatically labelled.

not employing labelling of probe include  
restriction fragment length polymorphisms,  
using PCR, RNAase cleavage and allele specific  
a probing.

ay employ the standard Southern blotting  
instance DNA may be extracted from cells and  
ifferent restriction enzymes. Restriction  
en be separated by electrophoresis on an  
ore denaturation and transfer to a  
ilter. Labelled probe may be hybridised to the  
the filter and binding determined. DNA for  
epared from RNA preparations from cells.  
experiments may be performed by hybridising  
cy conditions various probes to Southern  
ted with restriction enzymes. Suitable  
e achieved when a large number of hybridising  
ined while the background hybridisation was  
onditions nucleic acid libraries, e.g. cDNA  
ative of expressed sequences, may be

skilled in the art are well able to employ  
the desired stringency for selective  
into account factors such as  
and base composition, temperature and so

In some preferred embodiments of diagnostic assays according to the present invention, oligonucleotides according to the present invention that are fragments of any of the sequences shown in Figure 2, or any allele associated with disease resistance, e.g. as identified in Table 1, are at least about 10 nucleotides in length, more preferably at least about 15 nucleotides in length, more preferably at least about 20 nucleotides in length, more preferably about 30 nucleotides in length. Such fragments themselves individually represent aspects of the present invention. Fragments and other oligonucleotides may be used as primers or probes as discussed but may also be generated (e.g. by PCR) in methods concerned with determining the presence in a test sample of a sequence indicative of disease resistance.

There are various methods for determining the presence or absence in a test sample of a particular polypeptide, such as the polypeptide with the amino acid sequence shown in Figure 2, or other figure herein, or an amino acid sequence mutant, variant or allele thereof (e.g. including an alteration shown in Table 1).

A sample may be tested for the presence of a binding partner for a specific binding member such as an antibody (or mixture of antibodies), specific for one or more particular variants of the polypeptide shown in Figure 2, e.g. see Table 1.

In such cases, the sample may be tested by being contacted

th a specific binding member such as an antibody under appropriate conditions for specific binding, before binding is determined, for instance using a reporter system as discussed. If a panel of antibodies is used, different reporting labels can be employed for each antibody so that binding of each can be determined.

A specific binding member such as an antibody may be used to isolate and/or purify its binding partner polypeptide from a sample, to allow for sequence and/or biochemical analysis of the polypeptide to determine whether it has the sequence or properties of the wild-type polypeptide or a particular mutant, variant or allele thereof. Amino acid sequence is known in the art using automated sequencing machines.

The use of diagnostic tests for *mlo* alleles allows the breeder or plant breeder to establish, with full confidence independent from time consuming resistance tests, whether a desired allele is present in the plant of interest (or any thereof), whether the plant is a representative of a population of other genetically identical plants (e.g. an inbred variety or cultivar) or one individual in a sample of individuals (e.g. breeders' selection) or unrelated plants. The *mlo* alleles conferring the desirable disease resistance are recessive, and are not therefore detectable at the plant phenotype level when in a heterozygous condition in the presence of a wild-type *Mlo* allele. Diagnostic screening for the presence of such recessive alleles

is therefore only possible on material homozygous for the *mlo* locus and so delays substantially the generation in a plant breeding programme at which selection can be reliably and cost-effectively applied. In a backcross breeding programme where, for example, a breeder is aiming to introgress a desirable *mlo* allele into an elite adapted high performing target genotype, the *mlo* locus will be permanently in the heterozygous condition until selfing is carried out. Nucleic acid or polypeptide testing for the presence of the recessive allele avoids the need to test selfed progeny of backcross generation individuals, thus saving considerable time and money. In other types of breeding scheme based on selection and selfing of desirable individuals, nucleic acid or polypeptide diagnostics for the desirable *mlo* alleles in high throughput, low cost assays as provided by this invention, reliable selection for the desirable *mlo* alleles can be made at early generations and on more material than would otherwise be possible. This gain in reliability of selection plus the time saving by being able to test material earlier and without costly resistance phenotype screening is of considerable value in plant breeding.

By way of example for nucleic acid testing, the barley *mlo-5* resistance allele is characterized by a G- to A-nucleotide substitution in the predicted start codon of the *Mlo* gene (Table 1). The mutation may easily be detected by standard PCR amplification of a *Mlo* gene segment from genomic template DNA with the primers:

forward primer: 5'-GTTGCCACACTTTGCCACG-3'

reverse primer: 5'-AAGCCAAGACGACAATCAGA-3'

(for example), followed by digestion with the restriction enzyme *PshA1*. This generates a cleaved amplified polymorphic sequences (CAPS) marker which may be displayed using

5 conventional agarose gel electrophoresis. Presence of a 769 bp fragment is indicative of the presence of the *mlo-5* allele.

The *mlo-9* resistance allele is characterized by a C- to T-nucleotide substitution (Table 1). This allele is of particular relevance since it is used frequently in breeding  
10 material. The mutational event may be easily detected using the primers:

forward primer 5'-GRRGCCACACTTTGCCACG-3'

reverse primer 5'-AAGCCAAGACGACAATCAGA-3'

(for example) and subsequent digestion of genomic amplification  
15 products with the restriction enzyme *Hha1*. This generates a CAPS marker which may be displayed by conventional agarose gel electrophoresis. The presence of a 374 bp fragment is indicative of the presence of *mlo-9*.

A third, particularly interesting allele is *mlo-12*,  
20 characterised by a substitution a residue 240, specifically a Phe240 to leucine replacement. This may result from a C720 to A substitution in the encoding nucleotide sequence (Table 1). This is the only currently documented *mlo* allele for which conclusive evidence is available that the altered protein  
25 retains residual wild-type activity (Hentrich, 1979, Arch. Züchtungsvorsch., Berlin 9, S. 283-291). *mlo-12* exhibits no detectable spontaneous cell death reaction but confers a

sufficient level of resistance to pathogens such as the powdery mildew fungus. *mlo-12* may therefore be the allele of choice in breeding programs if minimal pleiotropic effects (spontaneous cell death) are desirable after introgression of the *mlo* resistance in elite breeding lines. Furthermore, the molecular site of the amino acid substitution within the Mlo protein allows the design of alleles with a residual wild-type activity, and also the obtention of interacting and/or inhibitory molecules, reducing undesirable pleiotropic effects from a complete loss of function of the Mlo protein.

Nucleic acid-based determination of the presence or absence of *mlo* alleles may be combined with determination of the genotype of the flanking linked genomic DNA and other unlinked genomic DNA using established sets of markers such as RFLPs, microsatellites or SSRs, AFLPs, RAPDs etc. This enables the researcher or plant breeder to select for not only the presence of the desirable *mlo* allele but also for individual plant or families of plants which have the most desirable combinations of linked and unlinked genetic background. Such recombinations of desirable material may occur only rarely within a given segregating breeding population or backcross progeny. Direct assay of the *mlo* locus as afforded by the present invention allows the researcher to make a stepwise approach to fixing (making homozygous) the desired combination of flanking markers and *mlo* alleles, by first identifying individuals fixed for one flanking marker and then identifying progeny fixed on the other side of the *mlo* locus all the time

vidence that the desirable *mlo* allele is still

disclosure provides sufficient information for  
in the art to obtain genomic DNA sequence for  
existing *mlo* allele and devise a suitable  
or polypeptide-based diagnostic assay.

as to which this may be applied include, for  
-3, *mlo*-4, *mlo*-5, *mlo*-6, *mlo*-7, *mlo*-8, *mlo*-  
*mlo*-13, *mlo*-16, *mlo*-17, *mlo*-26 and *mlo*-28,  
quence information is provided herein (see  
able 1). In designing a nucleic acid assay  
the distinctive variation in sequence that  
rticular variant allele. Thus, the present  
an oligonucleotide fragment of a *mlo*  
ence which allows it to hybridise  
allele as compared with other *mlo* alleles.  
de spans a nucleotide at which a *mlo*  
may include the mutated nucleotide at or  
nd. Such an oligonucleotide may  
se or anti-sense strand. The variation  
ng sequence of the *mlo* gene, or may lie  
ice or in an upstream or downstream non-  
n disruption affects or is otherwise  
n *Mlo* that results in the mildew

widely but not exclusively used in

Jorgensen - *Euphytica* (1992) 63: 141-

152), *mlo*-11 is also used. Use of *mlo* mutants in practical breeding has largely been restricted to spring barley, because the spontaneous cell death response associated with many of the mutant alleles appears to represent a penalty to plant growth and performance when incorporated into high yielding winter barley genotypes. However different *mlo* alleles have different degrees of associated spontaneous cell death response, and thus some, either existing or newly created from mutagenesis programmes or isolated as spontaneous mutants, are more suitable than others for incorporation into winter barley backgrounds. The *mlo*-12 allele may be particularly suitable since no detectable pleiotropic effects occur despite conferring a sufficient level of pathogen resistance. The use of *mlo* based mildew resistance more widely in winter barleys will have significant value for barley growers as well as significant economic and environmental implications such as reduced use of fungicide inputs with their associated treatments. The provision of nucleic acid diagnostics as provided herein enables rapid and accurate deployment of new and existing *mlo* alleles into winter barley germplasm.

Plants which include a plant cell according to the invention are also provided, along with any part or propagule of, seed, selfed or hybrid progeny and descendants. A plant according to the present invention may be one which does not breed true in one or more properties. Plant varieties may be included, particularly registrable plant varieties according



to Plant Breeders' Rights. It is noted that a plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene, introduced into a cell of the plant or an ancestor thereof.

5 In addition to a plant, the present invention provides any clone of such a plant, seed, selfed or hybrid progeny and descendants, and any part of any of these, such as cuttings, seed. The invention provides any plant propagule, that is any part which may be used in reproduction or propagation, sexual  
10 or asexual, including cuttings, seed and so on. Also encompassed by the invention is a plant which is a sexually or asexually propagated off-spring, clone or descendant of such a plant, or any part or propagule of said plant, off-spring, clone or descendant.

15 A further aspect of the present invention provides a method of making a plant cell involving introduction of the sequence (e.g. as part of a suitable vector) into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides  
20 into the genome.

Following transformation of a plant cell a plant may be regenerated.

The invention further provides a method of modulating *Mlo*  
25 expression in a plant, which may modulate a defence response in the plant, comprising expression of a heterologous *Mlo* gene sequence (or mutant, allele, variant or homologue thereof, as

discussed) within cells of the plant. As discussed further herein, modulation or alteration of the level of constitutive defence response in a plant may be by way of suppression, repression or reduction (in the manner of wild-type *Mlo*) or  
5 promotion, stimulation, activation, increase, enhancement or augmentation (in the manner of mutant *mlo*). Activation or enhancement of the defence response may confer or increase pathogen resistance of the plant, especially resistance to powdery mildew and/or rust (such as yellow rust).

10 The term "heterologous" may be used to indicate that the gene/sequence of nucleotides in question have been introduced into said cells of the plant or an ancestor thereof, using genetic engineering, ie by human intervention. A transgenic plant cell, i.e. transgenic for the nucleic acid in question, may be provided. The transgene may be on an extra-genomic vector or incorporated, preferably stably, into the genome. A heterologous gene may replace an endogenous equivalent gene, ie one which normally performs the same or a similar function, or the inserted sequence may be additional to the endogenous gene or other sequence. An advantage of introduction of a heterologous gene is the ability to place expression of a sequence under the control of a promoter of choice, in order to be able to influence expression according to preference, such as under particular developmental, spatial or temporal control, or under control of an inducible promoter. Furthermore, mutants, variants and derivatives of the wild-type gene, e.g. with higher or lower activity than wild-type, may be used in

place of the endogenous gene. Nucleic acid heterologous, or exogenous or foreign, to a plant cell may be non-naturally occurring in cells of that type, variety or species. Thus, nucleic acid may include a coding sequence of or derived from a particular type of plant cell or species or variety of plant, placed within the context of a plant cell of a different type or species or variety of plant. A further possibility is for a nucleic acid sequence to be placed within a cell in which it or a homologue is found naturally, but wherein the nucleic acid sequence is linked and/or adjacent to nucleic acid which does not occur naturally within the cell, or cells of that type or species or variety of plant, such as operably linked to one or more regulatory sequences, such as a promoter sequence, for control of expression. A sequence within a plant or other host cell may be identifiably heterologous, exogenous or foreign.

Down-regulation of wild-type *Mlo* gene function leads to stimulation of a constitutive defence response. This may be achieved in a number of different ways, as illustrated below.

The nucleic acid according to the invention may be placed under the control of an inducible gene promoter thus placing expression under the control of the user.

In a further aspect the present invention provides a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention. As discussed, this enables control of expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a

construct into a plant cell and/or induction of expression of a construct within a plant cell, e.g. by application of a suitable stimulus, such as an effective exogenous inducer or endogenous signal.

5       The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus (which may be generated within a cell or provided exogenously). The nature of  
10   the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression  
15   is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon application of the relevant stimulus by an amount effective to alter a phenotypic characteristic.  
20   Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level  
25   which brings about the desired phenotype.

Suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level

in virtually all plant tissues (Benfey et al, (1990a) EMBO J 9: 1677-1684); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, eg inner phloem, flower primordia, branching points in root and shoot (Medford, J.I. (1992) *Plant Cell* 4, 1029-1039; Medford et al, (1991) *Plant Cell* 3, 359-370) and the *Arabidopsis thaliana* LEAFY promoter that is expressed very early in flower development (Weigel et al, (1992) *Cell* 69, 843-859).

An aspect of the present invention is the use of nucleic acid according to the invention in the production of a transgenic plant.

When introducing a chosen gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur. Finally, as far as plants are concerned the target cell type must be such that cells can be regenerated into whole plants.

Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable technology,

such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green et al. (1987) *Plant Tissue and Cell Culture*, Academic Press), electroporation (EP 290395, WO 8706614) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman et al. *Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228 (1990d) Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

*Agrobacterium* transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, et al. (1988) *Plant Cell Rep.* 7, 379-384; Zhang, et al. (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, et al. (1989) *Nature* 338, 274-276; Datta, et al. (1990) *Bio/Technology* 8, 736-740; Christou, et al. (1991) *Bio/Technology* 9, 957-962; Peng, et al. (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, et al. (1992) *Plant Cell Rep.* 11, 585-591; Li, et al. (1993) *Plant Cell Rep.* 12, 250-255; Rathore, et al. (1993) *Plant Molecular Biology* 21, 871-884; Fromm, et al. (1990)

*Bio/Technology* 8, 833-839; Gordon-Kamm, et al. (1990) *Plant Cell* 2, 603-618; D'Halluin, et al. (1992) *Plant Cell* 4, 1495-1505; Walters, et al. (1992) *Plant Molecular Biology* 18, 189-200; Koziel, et al. (1993) *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, et al. (1993) *Plant Physiology* 102, 1077-1084; Somers, et al. (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In particular, *Agrobacterium* mediated transformation is now emerging also as an highly efficient alternative transformation method in monocots (Hiei et al. (1994) *The Plant Journal* 6, 271-282).

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, et al. (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where *Agrobacterium* is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with *Agrobacterium* coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from

cells, tissues and organs of the plant. Available techniques are reviewed in Vasil et al., *Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications*, Academic Press, 1984, and Weissbach and  
5 Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person  
10 practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for  
15 plant regeneration.

In the present invention, expression may be achieved by introduction of the nucleotide sequence in a sense orientation. Thus, the present invention provides a method of modulation of a defence response in a plant, the method comprising causing or  
20 allowing expression of nucleic acid according to the invention within cells of the plant. Generally, it will be desirable to stimulate the defence response, and this may be achieved by disrupting *Mlo* gene function.

Down-regulation of expression of a target gene may be  
25 achieved using anti-sense technology or "sense regulation" ("co-suppression").

In using anti-sense genes or partial gene sequences to



down-regulate gene expression, a nucleotide sequence is placed under the control of a promoter in a "reverse orientation" such that transcription yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene.

5 See, for example, Rothstein et al, 1987; Smith et al, (1988) *Nature* 334, 724-726; Zhang et al, (1992) *The Plant Cell* 4, 1575-1588, English et al., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and Flavell, (1994) *PNAS USA* 91, 3490-  
10 3496.

An alternative is to use a copy of all or part of the target gene inserted in sense, that is the same, orientation as the target gene, to achieve reduction in expression of the target gene by co-suppression. See, for example, van der Krol  
15 et al., (1990) *The Plant Cell* 2, 291-299; Napoli et al., (1990) *The Plant Cell* 2, 279-289; Zhang et al., (1992) *The Plant Cell* 4, 1575-1588, and US-A-5,231,020.

The complete sequence corresponding to the coding sequence (in reverse orientation for anti-sense) need not be used. For  
20 example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding sequence to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG  
25 codon, and perhaps one or more nucleotides upstream of the initiating codon. A further possibility is to target a conserved sequence of a gene, e.g. a sequence that is

characteristic of one or more genes, such as a regulatory sequence. Antisense constructs may involve 3' end or 5' end sequences of *Mlo* or homologues. In cases where several *Mlo* homologues exist in a plant species, the involvement of 5'- and 3'-end untranslated sequences in the construct will enhance specificity of silencing.

The sequence employed may be about 500 nucleotides or less, possibly about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, or about 100 nucleotides. It may be possible to use oligonucleotides of much shorter lengths, 14-23 nucleotides, although longer fragments, and generally even longer than about 500 nucleotides are preferable where possible, such as longer than about 600 nucleotides, than about 700 nucleotides, than about 800 nucleotides, than about 1000 nucleotides, than about 1200 nucleotides, than about 1400 nucleotides, or more.

It may be preferable that there is complete sequence identity in the sequence used for down-regulation of expression of a target sequence, and the target sequence, though total complementarity or similarity of sequence is not essential. One or more nucleotides may differ in the sequence used from the target gene. Thus, a sequence employed in a down-regulation of gene expression in accordance with the present invention may be a wild-type sequence (e.g. gene) selected from those available, or a mutant, derivative, variant or allele, by way of insertion, addition, deletion or substitution of one or more nucleotides, of such a sequence. The sequence need not

include an open reading frame or specify an RNA that would be translatable. It may be preferred for there to be sufficient homology for the respective anti-sense and sense RNA molecules to hybridise. There may be down regulation of gene expression even where there is about 5%, 10%, 15% or 20% or more mismatch between the sequence used and the target gene.

Generally, the transcribed nucleic acid may represent a fragment of an Mlo gene, such as including a nucleotide sequence shown in Figure 2, or the complement thereof, or may be a mutant, derivative, variant or allele thereof, in similar terms as discussed above in relation to alterations being made to a coding sequence and the homology of the altered sequence. The homology may be sufficient for the transcribed anti-sense RNA to hybridise with nucleic acid within cells of the plant, though irrespective of whether hybridisation takes place the desired effect is down-regulation of gene expression.

Anti-sense regulation may itself be regulated by employing an inducible promoter in an appropriate construct.

Constructs may be expressed using the natural promoter, by a constitutively expressed promoter such as the CaMV 35S promoter, by a tissue-specific or cell-type specific promoter, or by a promoter that can be activated by an external signal or agent. The CaMV 35S promoter but also the rice actin1 and maize ubiquitin promoters have been shown to give high levels of reporter gene expression in rice (Fujimoto *et al.*, (1993) *Bio/Technology* 11, 1151-1155; Zhang, *et al.*, (1991) *Plant Cell* 3, 1155-1165; Cornejo *et al.*, (1993) *Plant Molecular Biology*

23, 567-581).

For use in anti-sense regulation, nucleic acid including a nucleotide sequence complementary to a coding sequence of a *Mlo* gene (i.e. including homologues), or a fragment of a said  
5 coding sequence suitable for use in anti-sense regulation of expression, is provided. This may be DNA and under control of an appropriate regulatory sequence for anti-sense transcription in cells of interest.

Thus, the present invention also provides a method of  
10 conferring pathogen resistance on a plant, the method including causing or allowing anti-sense transcription from heterologous nucleic acid according to the invention within cells of the plant.

The present invention further provides the use of the  
15 nucleotide sequence of Figure 2 or a fragment, mutant, derivative, allele, variant or homologue thereof, such as any sequence shown or identified herein, for down-regulation of gene expression, particularly down-regulation of expression of an *Mlo* gene or homologue thereof, preferably in order to confer  
20 pathogen resistance on a plant.

When additional copies of the target gene are inserted in sense, that is the same, orientation as the target gene, a range of phenotypes is produced which includes individuals where over-expression occurs and some where under-expression of  
25 protein from the target gene occurs. When the inserted gene is only part of the endogenous gene the number of under-expressing individuals in the transgenic population increases. The

mechanism by which sense regulation occurs, particularly down-regulation, is not well-understood. However, this technique is well-reported in scientific and patent literature and is used routinely for gene control. See, for example, van  
5 der Krol et al., (1990) *The Plant Cell* 2, 291-229; Napoli et al., (1990) *The Plant Cell* 2, 279-289; Zhang et al, 1992 *The Plant Cell* 4, 1575-1588.

Again, fragments, mutants and so on may be used in similar terms as described above for use in anti-sense regulation.

9 Thus, the present invention also provides a method of conferring pathogen resistance on a plant, the method including causing or allowing expression from nucleic acid according to the invention within cells of the plant. This may be used to suppress Mlo activity. Here the activity of the product is  
5 preferably suppressed as a result of under-expression within the plant cells.

As noted, Mlo down-regulation may promote activation of a defence response, which may in turn confer or augment pathogen resistance of the plant, especially resistance to powdery  
1 mildew and/or rust (e.g. yellow rust).

Thus, the present invention also provides a method of modulating Mlo function in a plant, the method comprising causing or allowing expression from nucleic acid according to the invention within cells of the plant to suppress endogenous  
Mlo expression.

Modified versions of Mlo may be used to down-regulate endogenous Mlo function. For example mutants, variants,

derivatives etc., may be employed. For instance, expression of a *mlo* mutant sequence at a high level may out-compete activity of endogenous *Mlo*.

Reduction of *Mlo* wild type activity may be achieved by  
5 using ribozymes, such as replication ribozymes, e.g. of the hammerhead class (Haseloff and Gerlach, 1988, *Nature* 334: 585-591; Feyter et al. *Mol.*, 1996, *Gen. Genet.* 250: 329-338).

Another way to reduce *Mlo* function in a plant employs transposon mutagenesis (reviewed by Osborne et al., (1995)  
10 *Current Opinion in Cell Biology* 7, 406-413). Inactivation of genes has been demonstrated via a 'targeted tagging' approach using either endogenous mobile elements or heterologous cloned transposons which retain their mobility in alien genomes. *Mlo* alleles carrying any insertion of known sequence could be  
15 identified by using PCR primers with binding specificities both in the insertion sequence and the *Mlo* homologue. 'Two-element systems' could be used to stabilize the transposon within inactivated alleles. In the two-element approach, a T-DNA is constructed bearing a non-autonomous transposon containing  
20 selectable or screenable marker gene inserted into an excision marker. Plants bearing these T-DNAs are crossed to plants bearing a second T-DNA expressing transposase function. Hybrids are double-selected for excision and for the marker within the transposon yielding F<sub>2</sub> plants with transposed elements. The  
25 two-element approach has a particular advantage with respect to *Ac/Ds* of maize, as the transposed *Ds* is likely to be unlinked to the transposase, facilitating outcrossing and stabilization

es et al., (1994) *Science* 266, 789-793;  
urrent Opinion in Cell Biology 7, 406-

y mildew resistance is caused by the  
ild type allele, resulting in a  
otype. Substances that inhibit the  
ype protein may be used to induce the

complete inactivation of *Mlo*  
and may even be detrimental is  
of mutagen-induced *mlo* resistance  
have retained residual wild type  
les exhibit no detectable  
ich negatively affects  
ld (Hentrich, W (1979) *Arch.*  
. 283-291).

cted to be membrane-anchored by  
see e.g. Figure 7). This  
reinforced by recent analysis of  
*bidopsis thaliana*. Structure  
*thaliana* homologue also suggests  
brane helices. A comparison of  
addition conserved cysteine  
ellular loops 1 and 3 and high  
lices in the second  
he predicted transmembrane

helices 3 and 4. These conserved structural motifs in the family of Mlo proteins are reminiscent of G protein coupled receptors (GPCR) described extensively in mammalian systems. GPCRs are known to be activated by ligands and to amplify  
5 signals intracellularly via heterotrimeric G proteins. Without in any way providing a limitation on the nature or scope of any aspect of the present invention, it is predicted that Mlo activates an inhibitory G alpha subunit of heterotrimeric G proteins, thus leading to a downregulation of as yet unknown  
10 effector proteins.

The provision herein of Mlo sequence information enables the identification of antagonists of function of the Mlo protein (e.g. GPCR function). Antagonists of Mlo may block receptor activation by its unknown genuine ligand, mimicking  
15 recessive mutations in the Mlo gene. Such Mlo antagonists may be used as crop protection compounds, for example applied externally to the plant or crop or, where the compound is peptidyl in nature, delivered internally via a biological vector (e.g. recombinant infecting viral particle expressing  
20 the antagonistic molecule within target plant cells) or via a transgenic route (plants or plant cells genetically modified to express the antagonist molecule, perhaps under control of a promoter inducible by an externally applied compound (eg GST-II promoter from maize - Jepson et al Plant Molecular Biology  
25 26:1855-1866 (1994)) allowing control over the timing of expresion of the mlo inactivation phenotype.

Leaf segments of *Mlo* wild type plants may be tested with a



test substance, e.g. from a random or combinatorial compound library, for resistance upon challenge with pathogen such as powdery mildew. The detached leaf segment assay is used as a standard test system to score for susceptibility/resistance upon inoculation with powdery mildew spores. Leaf segments of 7-day-old seedlings of the genotype *Mlo RorI* may be placed on agar, for example individual wells of 96-well microtiter plates containing 50  $\mu$ l agar. Different compounds may be applied to the agar surface in each well at a concentration of about 1ppm dissolved in DMSO. Around seven days after inoculation of the detached leaf segments with pathogen, such as spores of a virulent powdery mildew isolate, compounds which induce resistance may be recognised by the absence of fungal mycelium on leaf segments in the microtiter plates.

A further selection may be used to discriminate between compounds that act in the *mlo* pathway and those that confer resistance by other mechanisms, or those which exhibit a direct fungitoxic activity. For this purpose mutants in genes (*Ror* genes) which may be required for *mlo* resistance (Freialdenhoven et al., (1996), The Plant Cell 8, 5-14) may be used. Mutants of these genes confer susceptibility to powdery mildew attack despite the presence of mlo resistance alleles. Plants of the genotype *Mlo rorl* (wild type *Mlo* protein and defective *Rorl* gene) may be used, for example, to test compounds which induce resistance on *Mlo Rorl* genotypes but exhibit susceptibility on the *Mlo rorl* genotype, enabling selection of candidate *Mlo* antagonists. Testing candidate compounds identified using a

leaf segment test may be used to drastically reduce the number of candidate compounds for further *in vitro* tests.

A further selection step of candidate antagonists may involve heterologous expression of the Mlo protein or a  
5 fragment thereof (e.g. in a baculovirus insect cell system) and subsequent binding assays with labelled molecules. Specific binding of compounds to cell lines expressing wild type Mlo protein is a good indicator of their antagonistic mode of action. Analysis of the deduced Mlo protein sequence has  
10 provided strong evidence that the protein is anchored in the membrane via seven transmembrane helices and may represent a novel member of the so-called serpentine receptor family. The conclusion is supported by the sequence data derived from homologous genes identified in barley, rice and *Arabidopsis*.  
15 Seven transmembrane proteins have been shown to be expressed at high level in the Baculovirus/insect cell system (up to  $10^7$  molecules per cell - Tate and Grisshamer, 1996, *TIBTECH* 14: 426-430). Since the family of Mlo proteins appears to be restricted to the plant kingdom, this provides a low-background  
20 environment for compound tests. Candidate compounds which are labelled, radioactively or non-radioactively, may be tested for specific binding to Sf9 insect cells expressing the Mlo protein after infection with a recombinant baculovirus construct. Specificity of the binding may be tested further by Sf9  
25 expression of mutant *mlo* proteins which carry characterised mutations (e.g. as in Table 1) leading *in vivo* to resistance.

Thus, in various further aspects the present invention relates to assays for substances able to interfere with Mlo function, i.e. confer a *mlo* mutant phenotype, such substances themselves and uses thereof.

5       The use of Mlo in identifying and/or obtaining a substance which inhibits Mlo function is further provided by the present invention, as is the use of Mlo in identifying and/or obtaining a substance which induces pathogen resistance in a plant.

10       Agents useful in accordance with the present invention may be identified by screening techniques which involve determining whether an agent under test inhibits or disrupts Mlo function to induce an *mlo* phenotype. Candidate inhibitors are substances which bind Mlo.

15       It should of course be noted that references to "Mlo" in relation to assays and screens should be taken to refer to homologues, such as in other species, including rice and wheat, not just in barley, also appropriate fragments, variants, alleles and derivatives thereof. Assessment of whether a test  
20       substance is able to bind the Mlo protein does not necessarily require the use of full-length Mlo protein. A suitable fragment may be used (or a suitable analogue or variant thereof).

25       Suitable fragments of Mlo include those which include residues known to be crucial for Mlo function as identified by *mlo* mutant alleles (Table 1). Smaller fragments, and analogues and variants of this fragment may similarly be employed, e.g.

as identified using techniques such as deletion analysis or alanine scanning.

Furthermore, one class of agents that can be used to disrupt Mlo activity are peptides fragments of it. Such peptides tend to be short, and may be about 40 amino acids in length or less, preferably about 35 amino acids in length or less, more preferably about 30 amino acids in length, or less, more preferably about 25 amino acids or less, more preferably about 20 amino acids or less, more preferably about 15 amino acids or less, more preferably about 10 amino acids or less, or 9, 8, 7, 6, 5 or less in length. The present invention also encompasses peptides which are sequence variants or derivatives of a wild type Mlo sequence, but which retain ability to interfere with Mlo function, e.g. to induce an *mlo* mutant phenotype. Where one or more additional amino acids are included, such amino acids may be from Mlo or may be heterologous or foreign to Mlo. A peptide may also be included within a larger fusion protein, particularly where the peptide is fused to a non-Mlo(i.e. heterologous or foreign) sequence, such as a polypeptide or protein domain.

Peptides may be generated wholly or partly by chemical synthesis. The compounds of the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois

1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

Another convenient way of producing a peptidyl molecule according to the present invention (peptide or polypeptide) is to express nucleic acid encoding it, by use of nucleic acid in an expression system, as discussed elsewhere herein. This allows for peptide agents to be delivered to plants transgenically, by means of encoding nucleic acid. If coupled to an inducible promoter for expression under control of the user, this allows for flexibility in induction of an *mlo* phenotype and pathogen resistance. This may allow for any side-effects arising from interference with Mlo function to be moderated.

In one general aspect the present invention provides an assay method for a substance able to interact with the relevant region of Mlo, the method including:

(a) bringing into contact a Mlo polypeptide or peptide fragment thereof, or a variant, derivative or analogue

thereof, and a test compound; and

(b) determining interaction or binding between said polypeptide or peptide and the test compound.

A test compound found to interact with the relevant  
5 portion of Mlo may be tested for ability to modulate, e.g. disrupt or interfere with, Mlo function, as discussed already above.

Another general aspect of the present invention provides  
10 an assay method for a substance able to induce an *mlo* mutant phenotype in a plant, the method including:

(a) bringing into contact a plant or part thereof (e.g. leaf or leaf segment) and a test compound; and

(b) determining Mlo function and/or pathogen resistance  
15 and/or stimulation of a defence response in the plant.

Susceptibility or resistance to a pathogen may be determined by assessing pathogen growth, e.g. for powdery mildew the presence or absence, or extent, of mycelial growth.

Binding of a test compound to a polypeptide or peptide may  
20 be assessed in addition to ability of the test compound to stimulate a defence response in a plant. Such tests may be run in parallel or one test may be performed on a substance which tests positive in another test.

25 Of course, the person skilled in the art will design any appropriate control experiments with which to compare results obtained in test assays.

Performance of an assay method according to the present invention may be followed by isolation and/or manufacture and/or use of a compound, substance or molecule which tests positive for ability to modulate Mlo function and/or induce  
5 pathogen resistance, such as resistance to powdery mildew.

The precise format of an assay of the invention may be varied by those of skill in the art using routine skill and knowledge. For example, interaction between substances may be  
10 studied *in vitro* by labelling one with a detectable label and bringing it into contact with the other which has been immobilised on a solid support. Suitable detectable labels, especially for peptidyl substances include <sup>35</sup>S-methionine which may be incorporated into recombinantly produced peptides and  
15 polypeptides. Recombinantly produced peptides and polypeptides may also be expressed as a fusion protein containing an epitope which can be labelled with an antibody.

An assay according to the present invention may also take the form of an *in vivo* assay. The *in vivo* assay may be  
20 performed in a cell line such as a yeast strain or mammalian cell line in which the relevant polypeptides or peptides are expressed from one or more vectors introduced into the cell.

For example, a polypeptide or peptide containing a fragment of Mlo or a peptidyl analogue or variant thereof as  
25 disclosed, may be fused to a DNA binding domain such as that of the yeast transcription factor GAL 4. The GAL 4 transcription factor includes two functional domains. These domains are the

DNA binding domain (GAL4DBD) and the GAL4 transcriptional activation domain (GAL4TAD). By fusing such a polypeptide or peptide to one of those domains and another polypeptide or peptide to the respective counterpart, a functional GAL 4

5 transcription factor is restored only when two polypeptides or peptides of interest interact. Thus, interaction of the polypeptides or peptides may be measured by the use of a reporter gene probably linked to a GAL 4 DNA binding site which is capable of activating transcription of said reporter gene.

10 This assay format is described by Fields and Song, 1989, Nature 340; 245-246. This type of assay format can be used in both mammalian cells and in yeast. Other combinations of DNA binding domain and transcriptional activation domain are available in the art and may be preferred, such as the LexA DNA

15 binding domain and the VP60 transcriptional activation domain.

When looking for peptides or other substances which interact with Mlo, the Mlo polypeptide or peptide may be employed as a fusion with (e.g.) the LexA DNA binding domain, with test polypeptide or peptide (e.g. a random or

20 combinatorial peptide library) as a fusion with (e.g.) VP60. An increase in reporter gene expression (e.g. in the case of  $\beta$ -galactosidase a strengthening of the blue colour) results from the presence of a peptide which interacts with Mlo, which interaction is required for transcriptional activation of the

25  $\beta$ -galactosidase gene.

The amount of test substance or compound which may be



added to an assay of the invention will normally be determined by trial and error depending upon the type of compound used. Typically, from about 0.001 nM to 1mM or more concentrations of putative inhibitor compound may be used, for example from 0.01  
5 nM to 100 $\mu$ M, e.g. 0.1 to 50  $\mu$ M, such as about 10  $\mu$ M. Greater concentrations may be used when a peptide is the test substance. Even a molecule which has a weak effect may be a useful lead compound for further investigation and development.

Compounds which may be used may be natural or synthetic  
10 chemical compounds used in drug screening programmes. Extracts of plants which contain several characterised or uncharacterised components may also be used. Antibodies directed to M10 or a fragment thereof form a further class of putative inhibitor compounds. Candidate inhibitor antibodies  
15 may be characterised and their binding regions determined to provide single chain antibodies and fragments thereof which are responsible for disrupting the interaction. Other candidate inhibitor compounds may be based on modelling the 3-dimensional structure of a polypeptide or peptide fragment and using  
0 rational drug design to provide potential inhibitor compounds with particular molecular shape, size and charge characteristics. It is worth noting, however, that combinatorial library technology provides an efficient way of testing a potentially vast number of different substances for  
5 ability to interact with and/or modulate the activity of a polypeptide. Such libraries and their use are known in the art, for all manner of natural products, small molecules and

peptides, among others. The use of peptide libraries may be preferred in certain circumstances.

Following identification of a substance or agent which  
5 modulates or affects *Mlo* function, the substance or agent may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition for inducing pathogen resistance in a plant. These may be applied to plants, e.g. for inducing pathogen  
10 resistance, such as resistance to powdery mildew. A further aspect of the present invention provides a method of inducing pathogen resistance in a plant, the method including applying such a substance to the plant. A peptidyl molecule may be applied to a plant transgenically, by expression from encoding  
15 nucleic acid, as noted.

A polypeptide, peptide or other substance able to modulate or interfere with *Mlo* function, inducing pathogen resistance in a plant as disclosed herein, or a nucleic acid molecule  
20 encoding a peptidyl such molecule, may be provided in a kit, e.g. sealed in a suitable container which protects its contents from the external environment. Such a kit may include instructions for use.

25 Further aspects and embodiments of the present invention will be apparent to those skilled in the art. The present invention will now be exemplified by way of illustration with

reference to the following figures:

Figure 1 Positional Cloning of *Mlo*. The *Mlo* locus has been mapped with increasing precision on the long arm of barley chromosome 4 using morphological, RFLP and AFLP markers. The upper part of the figure presents the genetic linkage maps of these markers relative to *Mlo*. All genetic distances are indicated in centiMorgan (cM) based on multi-point linkage analysis except for genetic distances between AFLP markers which are calculated by two-point-estimates. The morphological marker map (Jørgensen, 1977) positions *Mlo* at a distance of more than 20 cM to hairy leaf sheath (*Hs*) and glossy sheath/spike (*gsl*). The RFLP marker map is based on the analysis of 257  $F_2$  individuals derived from the cross Carlsberg II *Mlo* Grannenlose Zweizeilige *mlo*-11. The previously published RFLP map (Hinze et al., 1991) of the same cross was based on only 44  $F_2$  individuals. The gene was delimited to a 2.7 cM interval bordered by markers bAO11 and bAL88. AFLP markers were identified and mapped as described in Experimental procedures. Their genetic distance to *Mlo* is based on the cross Ingrid *Mlo* x BC<sub>7</sub>Ingrid *mlo*-3. The crucial result of the AFLP analysis has been the identification of two markers, Bpm2 and Bpm9, defining an 0.64 cM interval containing the *Mlo* locus and one marker (Bpm16) cosegregating with *Mlo* on the basis of more than 4,000 meiotic events. Marker Bxm2 which is located 0.1 cM telomeric to *Mlo* was derived from BAC F15 template DNA (see below). One YAC clone, YAC YHV303-A6, containing the

cosegregating marker Bpm16 and two flanking loci (Bpm2 and Bpm9), is shown in the middle section of the figure. The position of marker Bpm9 was only roughly estimated within the YAC clone as indicated by the arrow. The insert of BAC F15 represents a 60 kb subfragment of this YAC as indicated in the lower part of the Figure. After the identification of AFLP marker Bpm2 in BAC F15, marker Bxm2 was discovered and positioned 0.1 cM in telomeric orientation of *Mlo*. The approximate physical position of AFLP markers Bpm2, Bpm16, and Bxm2 (spanning an interval of approximately 30 kb) as well as the location of some rare occurring restriction sites are indicated. Dashed lines below the schematic representation of BAC F15 DNA show the position of the largest established DNA sequence contigs. The structure of the *Mlo* gene is given schematically in the bottom line of the Figure. Exons are highlighted by black boxes. Positions of mutational events are indicated for the eleven tested *mlo* alleles. Mutant alleles carrying deletions in their nucleotide sequence are marked with a  $\Delta$ ; the remaining mutant alleles represent single nucleotide substitutions resulting in amino acid exchanges in each case.

Figure 2 shows an *Mlo* coding sequence and encoded amino acid sequence according to the present invention. The amino acid sequence predicted from DNA sequences of RT-PCR products from Ingrid *Mlo* are shown. Nucleotide numbers are given according to translational start site.

Figure 3 Northern Blot Analysis of *Mlo* Transcript Accumulation. Total RNA (20  $\mu$ g) and poly(A)<sup>+</sup> RNA (5  $\mu$ g) of

seven-day-old uninfected barley primary leaves of one wild type (cultivar Ingrid *Mlo*) and two mutant (BC Ingrid *mlo*-1, BC Ingrid *mlo*-3) cultivars were isolated, separated on a 1.2% formaldehyde gel and transferred to a nitrocellulose membrane (Hybond). The filter was probed under stringent conditions (Sambrook et al., 1989) with the radioactivity labelled full size RT-PCR product derived from Ingrid *Mlo* (Figure 7). A clear signal is detected only in the lanes containing poly(A)<sup>+</sup> RNA. The signal corresponds to a size of approximately 2 kb.

Figure 4 Southern Blot Analysis of Intragenic Recombinants derived from *mlo* heteroallelic crosses. The alleles of two RFLP markers flanking *Mlo* on opposite sides of either susceptible  $F_2$  individuals or homozygous susceptible and homozygous resistant progeny were determined by Southern blot analysis. Plant DNA (10  $\mu$ g) of the individuals were digested with *Pst* I (A) or *Hae* III (B) and hybridized with the radioactively labelled RFLP markers WG114 (upper panel; maps 3.1 cM in centromeric orientation to *Mlo*; see Figure 1) and ABG366 (lower panel; maps 0.7 cM in telomeric orientation to *Mlo*; see Figure 1) according to standard procedures (Sambrook et al., 1989).

A DNA of the parental lines *mlo*-8 and *mlo*-1 and two homozygous susceptible (S, *Mlo Mlo*) and two resistant (R, *mlo mlo*) progenies derived from two susceptible  $F_2$  plants (designated 1 and 2) were tested. The DNAs in lanes S and R represent selection  $F_3$  individuals from  $F_3$  families obtained by selfing the susceptible  $F_2$  individuals 1 and 2. Note that

susceptible  $F_2$  individuals are expected to be heterozygous at *Mlo* in this section scheme. Infection phenotypes were scored seven days after inoculation with the *mlo* avirulent isolate K1. DNA from a third susceptible individual of this heteroallelic cross (see Table 7) is not included in this Figure.

B DNA of the parental lines *mlo-5* and *mlo-1* and seven homozygous susceptible (*S*, *Mlo Mlo*) and seven resistant (*R*, *mlo mlo*) progeny derived from seven susceptible  $F_2$  plants (designated 1 to 7) were tested. The DNAs in lanes *S* and *R* represent selected  $F_3$  individuals from  $F_3$  families obtained by selfing the susceptible  $F_2$  individuals 1 to 7. DNA was analyzed from two further susceptible individuals of this heteroallelic cross only in the  $F_2$  generation (8\* and 9\*).

Figure 5 shows an alignment of genomic sequences covering the barley *Mlo* gene and a rice homologue isolated via crosshybridization with a barley gene specific probe. The top line shows the barley *Mlo* genomic DNA sequence (exon sequences underlined). The bottom line shows the rice genomic sequence containing the rice *Mlo* homologue.

Figure 6 shows an alignment of genomic sequences carrying the barley *Mlo* gene and a barley homologue isolated via crosshybridization with a barley gene specific probe. The top line shows the barley *Mlo* genomic DNA sequence (exon sequences underlined). The bottom line shows the genomic sequence containing the barley *Mlo* homologue.

Figure 7 Nucleotide and Deduced Amino Acid Sequence of the Barley *Mlo* cDNA. The nucleotide and the deduced amino acid

sequence are based on the combined data of RT-PCR and RACE obtained from experiments using RNA of cultivar Ingrid Mlo. The stop codon is marked by an asterisk, the putative polyadenylation signal is underlined and the detected termini of RACE products are indicated by arrows above the sequence. Positions of introns as indentified by comparison with corresponding genomic clones are labelled by triangles below the nucleic acid sequence. Six predicted transmembrane spanning helices according to the MEMSAT algorithm (Jones et al., 1994) are boxed in grey colour. A putative nuclear localization signal (K-K-K-V-R) and casein kinase II site (S-I-F-D) in the carboxy-terminal half of the protein are shown in bold type.

Figure 8 shows genomic sequence of rice (*Oryza sativa*) homologue including coding and flanking sequences.

Figure 9 shows genomic sequence of barley (*Hordeum vulgare*) homologue including coding and flanking sequences.

Figure 10 shows cDNA sequence of rice homologue.

Figure 11 shows cDNA sequence of barley homologue.

Figure 12 shows cDNA sequence of *Arabidopsis thaliana* homologue.

Figure 13 shows amino acid sequence of rice homologue.

Figure 14 shows amino acid sequence of barley homologue.

Figure 15 shows amino acid sequence of *Arabidopsis* homologue.

Figure 16 shows a pretty box of amino acid sequences of Mlo, barley, rice and *Arabidopsis* homologues.

All documents mentioned in this document are incorporated by reference.

*EXAMPLE 1 - CLONING OF MLO OF BARLEY*

5

*Targeted search for AFLP markers tightly linked to Mlo*

Efforts to increase the DNA marker density around *Mlo* were coordinated with attempts to construct a local high resolution genetic map. An alternative possibility would have been to  
10 extend the population size of the characterized cross Carlsberg II *Mlo* x Grannenlose Zweizeilige *mlo-11* (Hinze et al., 1991) but it was felt to be advantageous to establish a high resolution map starting out from one of the available BC *mlo* lines and its recurrent parent line. Importantly, the  
15 donor parent of the BC line represents a different genetic background in comparison to the recurrent parent line. In this way, searching for linked AFLP markers could be started in parallel with generating a large mapping population from a cross between the same genetic lines. In addition, the BC line  
20 based cross allowed testing of colinearity of DNA markers in the vicinity of *Mlo* as determined from the cross Carlsberg II *Mlo* x Grannenlose Zweizeilige *mlo-11* (Hinze et al., 1991). For the new cross a *mlo-3* backcross (BC) line was used that had been backcrossed seven times into the genetic  
25 background Ingrid (BC<sub>7</sub> Ingrid *mlo-3* ; Hinze et al., 1991). The line was previously characterized to carry a relatively small introgressed DNA segment on barley chromosome 4. In addition,



the donor parent line Malteria Heda *mlo*-3 exhibits in comparison to DNA from the recurrent parent Ingrid polymorphisms with most of the identified RFLP loci linked to *Mlo*. Thus, by searching polymorphisms only between two DNA  
5 templates, from lines Ingrid *Mlo* and BC<sub>7</sub> Ingrid *mlo*-3, we hoped to increase the density of DNA markers with AFLPs around *Mlo* in a targeted manner.

The same two lines were crossed to establish a segregating population for high resolution mapping of DNA markers, formally  
10 representing an eighth backcross. F<sub>2</sub> individuals were scored for *mlo* resistance after powdery mildew inoculation with isolate K1 (virulent on Ingrid *Mlo* and avirulent on BC<sub>7</sub> Ingrid *mlo*-3 ). Initially, only a small fraction of the F<sub>2</sub> (77 individuals) was analyzed for recombination events with flanking RFLP markers.  
15 Analysis of four identified recombinants (designated 8-32-2, 7-38-4, 1-34-1, and 1-49-4) indicated colinearity of marker order in this cross compared to the previously analyzed cross Carlsberg II *Mlo* x Grannenlose Zweizeilige *mlo*-11 (Hinze et al., 1991). Several of the 77 F<sub>2</sub> seedlings which exhibited a  
20 susceptible phenotype and heterozygosity for the tested flanking DNA marker loci (bA011, bAL88/2, and bAP91; Hinze et al., 1991) were grown to maturity to provide further selfed seed material segregating for *Mlo/mlo*-3 in the F<sub>3</sub> generation. In total, leaf material was harvested for high resolution  
25 marker mapping from 2,026 individuals derived from either the selfed F<sub>2</sub> or F<sub>3</sub> generation.

AFLP marker candidates were identified by testing all

possible *Pst* I/*Mse* I primer combinations (1,024) extending into genomic sequences up to nucleotide positions +2 and +3, respectively. Similarly, almost 1,900 *Eco* RI/*Mse* I primer combinations (+3/+3) have been analyzed. Four DNA templates were included in this analysis: Ingrid *Mlo*, BC<sub>7</sub> Ingrid *mlo-3* , a DNA pool of two phenotypically *mlo* resistant F<sub>2</sub> individuals, and a DNA pool of nine phenotypically susceptible F<sub>2</sub> individuals. The resistant and susceptible F<sub>2</sub> individuals which were included as DNA pools in the AFLP search had been selected from the above mentioned RFLP analysis of 77 F<sub>2</sub> segregants. The pooled F<sub>2</sub> DNA enabled us to control whether candidate polymorphisms detected between template DNA from the parents were heritable traits in the F<sub>2</sub>. All identified AFLP candidate markers have been re-examined with eight DNA templates: Ingrid *Mlo*, BC<sub>7</sub> Ingrid *mlo-3* , DNA pools from individuals of three F<sub>3</sub> families which were phenotypically homozygous susceptible (*MloMlo*) according to K1 inoculation experiments; DNA of three resistant F<sub>2</sub> individuals. A total of 18 *Pst* I/*Mse* I and 20 *Eco* RI/*Mse* I primers were confirmed based on the selection procedure.

The number of identified AFLP markers made it useful to assign them first roughly to marker intervals based on the RFLP map around *Mlo*. It was hoped that this approach should enable both evaluation of the distribution of AFLPs among previously identified RFLP intervals close to *Mlo* and selection of a pair of flanking AFLP markers with which recombinants could be identified among the 2,026 segregants. For AFLP assignment we

used those four recombinants that had been identified with RFLP markers out of the above mentioned small sample of 77  $F_2$  segregants from Ingrid *Mlo* x BC<sub>7</sub> Ingrid *mlo-3* (two recombinants in interval bAP91-bAL88, one in *Mlo*-bAO11, and one in bAO11-ABG366). A total of 18 AFLPs were found to be located within a genetic distance of approximately 3.5 cM including *Mlo*.

*Construction of a high resolution AFLP map around Mlo*

A two-step procedure was used to construct the high resolution AFLP map. First, all 2,026 segregants were screened for recombination events between two AFLP markers on opposite sides of *Mlo* and subsequently only the few identified recombinants were used to map all the identified AFLPs in the 3.5 cM target interval. AFLP markers Bpm1 and Bpm9 were chosen, detecting each allelic DNA fragments in Ingrid *Mlo* and BC<sub>7</sub> Ingrid *mlo-3* and located on opposite sites of *Mlo* to screen DNA templates of the segregants for recombination events. Alternatively, the search for recombinants could have been carried out with the flanking RFLP markers bAO11 and bAL88. However, although the conversion into cleaved amplified polymorphic sites (CAPS) was successful for both markers, difficulties to display the alleles of both loci simultaneously from crudely purified genomic DNA were encountered. A total of 2,026 individuals ( $F_2$  or  $F_3$  segregants) were screened simultaneously with AFLP markers Bpm1 and Bpm9 and 98 recombinants were identified. AFLP analysis was subsequently carried out with each of the 98 DNA templates of the

recombinants to identify the alleles of each of the identified  
of AFLP loci. The recombinants have been selfed and  
inoculation experiments with powdery mildew isolate K1 were  
performed using at least 25 individuals of each recombinant  
5 family to deduce the alleles of the previous generation at the  
*Mlo* locus. The obtained data enabled the construction of a high  
resolution map around *Mlo* based on more than 4,000 meiotic  
events and a resolution of at least 0.025 cM derived via two-  
point estimates. The essential result has been the  
10 identification of a DNA marker cosegregating with *Mlo* (Bpm16)  
and two flanking markers (Bpm2 and Bpm9) at a distance of 0.25  
and 0.4 cM respectively (Figure 1).

*Construction of a large insert size barley YAC library,  
15 isolation of Bpm16 containing YACs, and physical delimitation  
of Mlo*

The genetic evidence indicates that *mlo* resistance is due  
to loss of function in the *Mlo* wild type allele. Therefore, it  
was decided to establish a large insert size YAC library from  
20 cultivar Ingrid *Mlo* into vector pYAC4 (Burke et al., 1987;  
Hieter, 1990). Megabase DNA suitable for YAC cloning  
experiments was prepared in mg amounts from mesophyll  
protoplasts of five-day-old seedlings according to a modified  
protocol described by Siedler and Graner (1991). The DNA was  
25 partially digested with *Eco* RI in the presence of *Eco* RI  
methyltransferase to obtain DNA fragments after preparative  
pulsed-field gel electrophoresis (PFGE) in the size range of

500-600 kb. After ligation with *Eco* RI digested pYAC4, the DNA was transformed into yeast strain AB1380 and colonies carrying recombinant pYAC4 DNA were selected on solidified synthetic complete medium lacking tryptophan and uracil (Sherman et al., 1986). Forty randomly selected yeast colonies were tested for the presence of barley DNA using labelled barley genomic DNA in Southern experiments. The size of the YAC inserts was found after PFGE separations to vary between 500 and 800 kb. On average a genetic distance of 0.2 cM was expected to be represented on the individual recombinant YAC clone. A total of ~40,000 clones representing four barley genome equivalents have been generated.

Four YAC clones (designated 303A6, 322G2, 400H11, and 417D1) have been isolated with marker Bpm16 cosegregating with *Mlo*. Their insert size was determined by PFGE to be 650, 710, 650, and 820 kb respectively. AFLP analysis had shown that three of these clones (303A6, 322G2, and 417D1) contain also both flanking marker loci whereas clone 400H11 contains only loci Bpm16 and Bpm2. These findings strongly suggested that the *Mlo* gene had been physically delimited on recombinant YAC clones 303A6, 322G2, and 417D1.

YAC 303A6 was chosen for subcloning experiments into BAC vector pEC5BAC4 containing a unique *Eco* RI site (Shizuya et al., 1992; the vector pEC5BAC4 is described by Frijters and Micheltore, 1996; submitted). Total yeast DNA of this clone was partially digested with *Eco* RI to obtain DNA fragments with an average size of 50 kb and ligated into *Eco* RI digested and

dephosphorylated BAC vector. Bacterial colonies containing YAC 303A6-derived DNA in pEC5BAC4 were identified by replica colony hybridization experiments. One set of colony containing membranes was hybridized with labelled yeast AB1380 DNA and the replica set was hybridized with labelled PFGE-purified YAC303A6 DNA. Recombinant BAC clones containing the AFLP locus Bpm16 were subsequently identified using the cloned 108 bp *Pst* I/*Mse* I genomic Bpm16 fragment as a probe in colony hybridization experiments.

One BAC clone, BAC F15, containing an insert of ~ 60 kb was chosen for further detailed studies. It was found that the recombinant BAC clone contained in addition the AFLP marker locus Bpm2, but not Bpm9. At this point the BAC F15 insert DNA indicated successful physical delimitation in telomeric orientation but it was an open question whether the insert would contain bordering sequences in centromeric direction. Instead of constructing a BAC contig between Bpm 16 and Bpm9, the option to develop new polymorphic markers from BAC F15 was chosen. An allelic *Xba* I/*Mse* I polymorphism (designated Bxm2) was identified between the parental lines Ingrid *Mlo* and BC<sub>7</sub> Ingrid *mlo*-3.

An analysis of the 25 recombinant individuals carrying recombination events within the *Mlo* containing interval Bpm2-Bpm9 enabled mapping of Bxm2 in centrometric orientation at a distance of 0.1 cM from *Mlo*. Only four out of the 16 available recombinants in the interval Bpm9-*Mlo* and none of the 9 recombinants in the interval *Mlo*-Bpm2 were found to exhibit a

recombination event between Bxm2 and Mlo. It was concluded that Mlo had been physically delimited on BAC F15 between marker loci Bpm2 and Bxm2 (Figure 1).

#### 5 Identification of the Mlo gene and mlo mutants

A random sequencing project was initiated to determine sequence contigs of the ~60 kb insert of BAC F15 before marker Bxm2 was identified and shown to delimit the gene in telomeric orientation. In parallel, a physical map was generated  
10 (Figure 1). The physical map indicated that the flanking markers Bpm2 and Bxm2 are physically separated by ~30 kb. The sequence contigs were searched for regions of high coding probability using the UNIX versions of the STADEN program package. Only one sequence contig of almost 6 kb, including the  
15 cosegregating marker Bpm16, revealed an extensive region of high coding probability.

RT-PCR reactions were performed with total leaf RNA derived from cultivar Ingrid Mlo using a series of primers deduced from regions which indicated high coding probabilities  
20 and obtained in each case a distinct amplification product. Sequencing of the largest RT-PCR products revealed a single extensive open reading frame of 1,602 bp (Figure 2). The deduced putative protein of 533 amino acids has a molecular weight of 60.4 kDal. The ~1.7 kb RT-PCR product was used as a  
25 hybridization probe and detected a single RNA transcript of ~1.9 kb length. (Figure 3). A comparison of the genomic sequence and the largest RT-PCR fragment reveals 12 exons and

11 introns, each flanked by the characteristic splice site sequences (Figure 1).

Because marker Bpm16 is located at the 3' end of the above  
5 described gene (exon 11) and cosegregates with the *Mlo* locus,  
we started a direct PCR sequencing of the various available  
mutagen-induced *mlo* resistance alleles. We identified in 14  
out of 15 tested mutant alleles nucleotide alterations which  
result either in single amino acid alterations, deletions or  
10 frame shifts of the wild type sequence (Table 1). We suspect  
that mutant allele *mlo*-2 is located within the promoter- or 5'  
untranslated sequences. The region is notoriously difficult to  
be sequenced via direct PCR sequencing from genomic DNA  
templates but experiments using a series of nested primers are  
15 likely to solve this problem. In summary, the comparative  
sequencing of genomic DNA from various mutant *mlo* lines and  
their respective *Mlo* wild type cultivars provided strong  
evidence that *Mlo* has been identified.

## 20 *Intragenic recombinants*

It had been the intention to provide a chain of evidence  
for the molecular isolation of *Mlo* which did not rely upon  
complementation experiments via transgenic barley plants. We  
had chosen to develop an unusual genetic tool to confirm that  
25 the identified gene represented *Mlo*. It was reasoned that if  
the mutations observed in the above described gene caused  
resistance to the powdery mildew fungus, recombination events



between mutant allele sites should restore wild type sequences. It was predicted that those intragenic recombinants would exhibit susceptibility upon powdery mildew attack.

A crossing scheme was devised involving *mlo* resistance alleles *mlo-1*, *mlo-5*, and *mlo-8*. The mutant alleles originate from the genetic backgrounds Haisa (*mlo-1*) and Carlsberg II (*mlo-5* and *mlo-8*). Intermutant crosses were performed as shown in Table 2 generating in each case at least 10  $F_1$  plants.  $F_2$  populations were obtained by self-fertilization.  $F_2$  seedlings were screened for rare susceptible individuals after inoculation with powdery mildew isolate K1 which is virulent on each of the parental *Mlo* wild type cultivars. Susceptible  $F_2$  individuals were identified with a frequency of  $\sim 6 \times 10^{-4}$ . In contrast, if comparable numbers of progenies from selfings of each of the *mlo* mutants were tested for resistance to K1, no susceptible seedling was identified. This finding strongly indicated that the majority of the susceptible individuals derived from the intermutant crosses were not due to spontaneous reversion events of the mutant *mlo* alleles.

Inheritance of the susceptible  $F_2$  individuals was tested after selfing in  $F_3$  families. Each of the  $F_2$  individuals segregated susceptible and resistant  $F_3$  individuals indicating heterozygosity for alleles conferring resistance/susceptibility in the  $F_2$ . Homozygous susceptible  $F_3$  progeny were isolated for the majority of susceptible  $F_2$  individuals by selfing of  $F_3$  individuals and subsequent identification of  $F_4$  families in which only susceptible individuals were detected.

A molecular analysis of the susceptible individuals has been performed using RFLP markers known to be tightly linked (< 3 cM) on each side of the *Mlo* locus (Figure 4). RFLP marker WG114 maps in centromeric orientation relative to *Mlo*, marker  
5 ABG366 maps in the direction of the telomere. Detected RFLP alleles are shown for the intermutant crosses *mlo-8* x *mlo-1* (A) and *mlo-1* x *mlo-5* (B). DNA was analyzed either from susceptible  $F_2$  individuals (indicated by \*) or from homozygous susceptible (S) and homozygous resistant (R)  $F_3$  progeny obtained from  
10 selfed susceptible  $F_2$  individuals.

The homozygous susceptible  $F_3$  progeny from the susceptible  $F_2$  plant #1 of cross *mlo-8* x *mlo-1* (Figure 4) reveals the WG114 allele derived from the *mlo-1* parent in centromeric orientation next to *Mlo* and the  
15 ABG366 allele from the *mlo-8* parent in telomeric orientation to *Mlo*. The homozygous resistant  $F_3$  progeny from  $F_2$  plant #1 of this cross reveals in contrast only the flanking marker alleles derived from parent *mlo-1*. The finding strongly suggested that susceptibility in  $F_2$  plant #1 is caused by a cross-over type of  
20 recombination in the preceding meiosis of one chromosome which results in a restoration of the *Mlo* wild type allele whereas the second  $F_2$  chromosome of individual 1 contains a functionally unaltered *mlo-1* allele. The allelotypes of the RFLP loci of the homozygous susceptible  $F_3$  progeny from  
25 susceptible  $F_2$  plant #2 are identical to the one described above. However, flanking marker alleles from the homozygous resistant  $F_3$  progeny of this individual are in both cases

derived from the *mlo-8* parent. It is concluded that again a cross-over type of recombination restored one *Mlo* wild type allele in the susceptible  $F_2$  individual #2.

Nine susceptible  $F_2$  individuals were recovered from the cross *mlo-1* x *mlo-5* (Figure 4). For susceptible  $F_2$  individuals #1 to #7 both homozygous susceptible and homozygous resistant  $F_3$  progeny were analyzed at the DNA level. Note that only DNA from the heterozygous susceptible  $F_2$  individuals was analyzed in the case of individuals #8 and #9 (marked by a \*). The following allele patterns with respect to the flanking RFLP loci were observed: (i) homozygous resistant  $F_3$  progeny showed on both sides of *Mlo* either only the allelotypes of loci WG114 and ABG366 derived from the *mlo-1* parent (individuals #1, #3, #6, #7) or only the allelotypes derived from the *mlo-5* parent (individuals #2, #4, #5). (ii) Homozygous susceptible  $F_3$  progeny showed in contrast either only the allelotypes of both loci derived from the *mlo-5* parent (no. #3, #5, #6) or they showed different allelotypes on both sides of *Mlo* (individuals #1, #2, #4, #7). (iii) The homozygous susceptible  $F_3$  progeny with different allelotypes on both sides always contain in centromeric orientation the *mlo-1* derived WG114 allele and in telomeric orientation the *mlo-5* derived ABG366 allele. (iv) The heterozygous susceptible  $F_2$  individual #8 reveals on either side next to *Mlo* only the alleles derived from parent *mlo-5*. The heterozygous susceptible individual #9 reveals in centromeric orientation alleles derived from both parents *mlo-1* and *mlo-5* whereas only the *mlo-5* derived allele is detected in

telomeric orientation. A comprehensive interpretation of the data suggests that susceptibility in  $F_2$  individuals no. #1, #2, #4, #7, and #9 is caused by a cross-over type of recombination restoring the *Mlo* wild type allele. Non cross-over types of recombination may have restored the *Mlo* wild type allele in individuals no. #3, #5, #6, and #8.

A compilation of the detected flanking RFLP alleles of all isolated susceptible  $F_2$  individuals or homozygous  $F_3$  progeny is shown in Table 3. Note that individual #3 of the cross *mlo-8* x *mlo-1* is not shown in Figure 4. The compilation reveals that (i) cross-over types of recombination (CO) and non cross-over types of recombination (NCO) are found with a ratio of 7 : 5, (ii) cross-over types of recombination are resolved unidirectional, and (iii) NCO recombinants were not observed with parental *mlo-1*-linked RFLP alleles.

The CO type intragenic recombinants isolated from heteroallelic *mlo* crosses were used to test whether wild type sequences of the *Mlo* candidate gene had been restored. For the three relevant alleles *mlo-1*, *mlo-5*, *mlo-8* alleles candidate mutation sites have been identified (Table 1 and 4). Direct PCR sequencing of genomic DNA of susceptible intragenic recombinants derived from both heteroallelic crosses *mlo-1* x *mlo-8* and *mlo-1* x *mlo-5* revealed restoration of wild type sequences (Table 4). This observation strongly suggests that the intragenic cross over event occurred between nucleotide -1 and +483 in the former and +3 and +483 in the latter cross (according to translational start site). Thus, the molecular

analysis of seven intragenic recombinants from two heteroallelic crosses provides final proof that the above described candidate gene represents *Mlo*.

5    **EXAMPLE 2 - HOMOLOGUES OF THE IDENTIFIED MLO GENE**

The available expressed sequence tag (EST) databases of *Oryza sativa* (rice) and *Arabidopsis thaliana* were searched for homologous protein sequences. Five *Arabidopsis* cDNA clones were  
10 identified whose deduced amino acid sequences show substantial similarity to the *Mlo* protein. Remarkable is cDNA clone 205N12T7 which reveals a chance probability of  $1.2 \times 10^{-45}$ . In addition, at least one significant homologue was found in rice (OSR16381A).

15        A rice BAC library (Wang et al., 1995) has also been screened with a labelled barley genomic fragment containing *Mlo*. A BAC clone containing an insert of ~23 kb was isolated. Subsequent subcloning enabled isolation of a 2.5 kb *Pst* I genomic rice fragment showing strong cross-hybridization with  
20 the barley *Mlo* gene probe. DNA sequencing of this fragment revealed remarkable DNA sequence similarities within exon sequences of the barley *Mlo* gene (Figure 5).

Finally, a 13 kb  $\lambda$  genomic barley clone derived from cultivar Igri (Stratagene) was isolated with a labelled barley  
25 genomic fragment containing *Mlo*. The nucleotide sequence derived from a subcloned 2.6 kb *Sac* I fragment reveals again extensive sequence similarities to the *Mlo* gene (Fig. 6). The

location of the barley *Mlo* homologue within the genome is not within BAC F15 DNA.

In summary, there is conclusive evidence for *Mlo* homologues both in a monocotyledonous and a dicotyledonous plant species.

#### *Discussion*

Any speculation as to mode of action of *Mlo* and *mlo* nucleic acid and polypeptides should provide no limitation on the nature or scope of any aspect or embodiment of the present invention.

In plants, resistance to pathogens is frequently determined by dominant resistance genes, whose products are assumed to recognize pathogen-derived avirulence gene products. This mode of pathogen defence follows Flor's gene-for-gene hypothesis (Flor, 1971). Recently, several 'gene-for-gene' type resistance genes have been molecularly isolated (Martin et al., 1993; Bent et al., 1994; Jones et al., 1994; Mindrinos et al., 1994; Whitham et al., 1994; Grant et al., 1995; Lawrence et al., 1995; Song et al., 1995). The surprising finding is that the deduced proteins share remarkable similar structural domains although they trigger resistance reactions to pathogens such as viruses, fungi, and bacteria (Dangl, 1995; Staskawicz et al., 1995). The isolated genes code for proteins that either contain a leucine-rich region (LRR), with or without an attached nucleotide binding site (NBS), indicative of ligand-binding and protein-protein interaction or encode a simple

serine/threonine kinase. A structural combination of LRR and the kinase domain has been reported in the deduced protein from the rice *Xa21* resistance gene (Song et al., 1995). The structural similarity of resistance genes in 'gene-for-gene' defence makes the existence of a common underlying resistance mechanisms likely.

Resistance mediated by recessive resistance alleles of the *Mlo* gene differs in various aspects from 'gene-for-gene' resistance (see introductory comments above). The molecular isolation of the *Mlo* gene and the sequencing of various mutation-induced *mlo* alleles described here, confirms previous interpretations from combined mutational and Mendelian genetic studies (Hentrich, 1979; Jørgensen, 1983). It is concluded that defective alleles of the *Mlo* locus mediate broad spectrum resistance to pathogens such as the powdery mildew pathogen. This is inconsistent with the involvement of a specific recognition event of a pathogen-derived product as has been proposed for race-specific resistance genes.

Pleiotropic effects of *mlo* alleles have provided some clues towards the development of a molecular concept of the observed broad spectrum resistance response.

Firstly, aseptically grown *mlo* plants exhibit at a high frequency a spontaneous formation of cell wall appositions (CWAs) in leaf epidermal cells (Wolter et al., 1993). Those CWAs are usually formed in response to attempted pathogen penetration directly beneath the fungal apressorium. CWAs are believed to form a physical barrier against pathogen ingress

and have been implicated repeatedly in *mlo* mediated resistance (Bayles, 1990).

Secondly, at a later stage, the plants develop macroscopically detectable leaf necrotic flecks. The spontaneous leaf necrosis response has been extensively studied with a unique collection of 95 chemically-induced *mlo* alleles (Hentrich, 1979). The alleles were classified as either showing a gradually different infection phenotype upon infection of a mixture of nine powdery mildew isolates. Those *mlo* alleles which give rise to an intermediate infection phenotype (i.e. development of a considerable number of sporulating fungal colonies upon inoculation) showed no detectable spontaneous leaf necrosis whereas the category of the most effective resistance alleles exhibits pronounced necrosis in the absence of the pathogen. Thus, there is solid evidence that the former category of *mlo* alleles retain residual wild type allele activity and those alleles appear to exhibit no detectable spontaneous leaf necrosis.

Thirdly, a constitutive expression of defence-related genes has been observed in *mlo* seedlings grown under mildew-free conditions - in primary leaves when 10-11 days old; this includes genes of the *PR-1* family, chitinases and peroxidases.

We have shown that *mlo* in barley confers increased resistance to different types of yellow rust (*Puccinia struiformis*) when a one to one mixture of talcum powder and spores were aviblown onto leaves of *mlo* barley plants after onset of constitutive expression of defence related genes (10-



11 day old *mlo* seedlings).

Thus, it appears that multiple defence-associated responses are constitutively expressed in *mlo* plants.

The temporal relationship of these events is interesting:

5 the onset of constitutive defence-related transcript accumulation is detected in 11 day-old seedlings and precedes CWA formation which is followed by the appearance of macroscopically visible leaf necrosis. Importantly, however, *mlo* resistance can be experimentally tested as early as in five  
10 day-old seedlings and is fully functional at this time. We conclude that the *Mlo* protein has a negative regulatory function in plant defence and that plants with a defective protein are 'primed' for the onset of defence responses.

The deduced amino acid sequence of *Mlo* reveals no  
15 significant homologies to any of the described plant resistance genes so far, supporting the idea of a distinct molecular resistance mechanism. The *Mlo* gene shows also no striking similarities to any characterized plant or mammalian gene sequence in the various data bases. However, highly significant  
20 homologous sequences have been identified in the EST and genomic databases both from rice and *Arabidopsis thaliana* (Table 5 and Figure 5). This strongly suggests that the *Mlo* protein represents a member of a novel protein family. A putative nuclear localization motif (NLS) is found within exon  
25 12 providing indication of nuclear localization of the protein (KEKKKVR; Nigg et al., 1991). The significance of this motif is supported by a casein kinase II motif located 14 amino acids

into direction of the NH<sub>2</sub>-terminus (SIFD; Rihs et al., 1991). Functional tests may examine the putative subcellular localization of the Mlo protein.

Mutations have been described also in other plant species in which defence responses to pathogens appear to be constitutively expressed (Walbot et al., 1983; Pryor, 1987; Jones, 1994). It has been suggested that this class of mutants, termed lesion mimics (*Les*) or necrotic mutants (*nec*), affect the control of plant defence responses. Recessively inherited lesion mimic mutants have been systematically analysed in *Arabidopsis thaliana* (Greenberg and Ausubel, 1993; Dietrich et al., 1994; Greenberg et al., 1994; Weymann et al. 1995). The affected genes have been designated *acd* (accelerated cell death; *acd1* and *acd2*) or *lsd* (lesions simulating disease resistance response; *lsd1* to *lsd7*).

Each of the mutants exhibits, in the absence of pathogens, HR characteristics such as plant cell wall modifications and the accumulation of defence-related gene transcripts. Leaves of the *acd2* mutant have been shown to accumulate high levels of salicylic acid and of the *Arabidopsis* phytoalexin, camelexin (Tsuji et al., 1992). Importantly, *acd* and *lsd* mutants exhibit elevated resistance to a bacterial (*P. syringae*) and fungal (*P. parasitica*) pathogen. The *lsd1* mutant is exceptional in that it confers heightened pathogen resistance at a prelesion state, in contrast to the other defective loci which exhibit elevated pathogen resistance only in the lesion-positive state. In this respect, *lsd1* resembles the *mlo* mutants in barley. Another

striking feature of *lsd1* is the indeterminate spread of lesions in contrast to the other mutants where lesion growth is determinate.

## 5 EXPERIMENTAL PROCEDURES

### *Plant Material*

A compilation of the *mlo* mutants and their mother varieties analyzed in this study has been described by  
10 Jørgensen (1992) [*mlo*-1, *mlo*-3, *mlo*-4, *mlo*-5, *mlo*-7, *mlo*-8, *mlo*-9, *mlo*-10, *mlo*-11] and by Habekuss and Hentrich (1988) [mutants in cultivar Plena 2018 (*mlo*-13), 2034 (*mlo*-17), 2118]. Since mutant 2118 has not been assigned to an allele number so far, we designate the allele here as *mlo*-26, according to  
15 current numbering in the GrainGene database  
([gopher://greengenes.cit.cornell.edu:70/77/.graingenes.ndx/index?mlo](http://gopher://greengenes.cit.cornell.edu:70/77/.graingenes.ndx/index?mlo)).

The high resolution map is based on a cross between Ingrid *Mlo* x BC<sub>7</sub> Ingrid *mlo*-3. F<sub>1</sub> plants were selfed generating a  
20 segregating F<sub>2</sub> population of approximately 600 plants. Phenotypically susceptible F<sub>2</sub> plants which showed heterozygosity for RFLP markers on opposite sites of *Mlo* were selfed and generated further segregants in the F<sub>3</sub> generation for high resolution mapping.

25

### *Powdery Mildew Infection Tests*

The fungal isolate K1 (Hinze et al., 1991) is virulent on

all cultivars used in this study carrying the *Mlo* allele and avirulent on all tested *mlo* genotypes. Plant growth and inoculation with *Erysiphe graminis* f sp *hordei* were carried out as described previously (Freialdenhoven et al., 1996). The genotype at *Mlo* of recombinants used for the high resolution map were determined after selfing and subsequent inoculation experiments in  $F_3$  or  $F_4$  families comprising at least 24 individuals.

#### 10 AFLP Analysis

Genomic DNA for AFLP analysis was isolated according to Stewart and Via (1993). AFLP analysis was carried out with minor modifications as described by Vos et al. (1995). For screening of AFLP markers linked to *Mlo* we used the enzyme combinations Pst I/Mse I with amplification primers carrying +2 and +3 selective bases respectively in genomic sequences of amplified fragments. For Eco RI/Mse I amplification primers we used +3 and +3 selective bases respectively. A set of four DNA templates has been used: from the susceptible parent cultivar Ingrid *Mlo*, the resistant parent BC<sub>7</sub>Ingrid *mlo*-3, a pool of two resistant  $F_2$  individuals (*mlo*-3 *mlo*-3) and a pool of nine susceptible  $F_2$  individuals (*Mlo Mlo*) derived from the cross Ingrid *Mlo* x BC<sub>7</sub> Ingrid *mlo*-3. Amplified genomic fragments representing AFLP markers Bpm2, Bpm9, and Bpm16 (Figure 1) were cloned and sequenced as follows: gel pieces (fixed by vacuum drying to Whatman 3MM paper) containing the amplified genomic fragments were identified via autoradiography and subsequently

excised. 100  $\mu$ l water were added, boiled for 10 min. and after centrifugation 5  $\mu$ l of the supernatant were used as a template for non-radioactive reamplification (30 cycles) with the selective AFLP primers. Amplification products were isolated  
5 after agarose gel using a DNA isolation kit (Jetsorb, Genomed Inc., USA). DNA was reated with Klenow polymerase and T4 polynucleotide kinase and subsequently cloned in the EcoRV site of pBluescript SK (Stratagene). Sequencing reactions were performed using a dye terminator cycle sequencing reaction kit  
10 (Perkin Elmer) and resolved either on an ABI 373 or 377 (Applied Biosystems) automated sequencer.

*Barley YAC Library and BAC Sublibrary Construction of YAC  
YHV303-A6*

15 The YAC library of barley cultivar Ingrid was established using the pYAC4 vector (Burke et al., 1987; Kuhn and Ludwig 1994) and yeast strain AB 1380. Details of the library construction and its characterization will be described elsewhere. Screening for YAC clones containing marker Bpm16  
20 was done by AFLP analysis. For construction of a BAC sublibrary of YAC YHV303-A6, total DNA of this yeast clone was used. After partial Eco RI digestion and preparative pulsed-field gel electrophoresis, DNA fragments in the size range of 50 kb were recovered and subcloned in the pECBAC4 vector.  
25 Clones carrying YHV303-A6 derived inserts were identified by a two-step colony hybridization procedure. First total labelled DNA of the non-recombinant yeast strain AB 1380 was used as a

probe to eliminate most of the clones carrying insert DNA derived from the host strain. In a subsequent hybridization step the remaining clones were probed with the labelled recombinant chromosome YHV303-A6 after enrichment by  
5 preparative pulsed-field gel electrophoresis.

#### *DNA Sequencing of BAC F15*

DNA of BAC F15 was isolated by an alkaline lysis large scale plasmid preparation according to Sambrook et al. (1989).  
10 50  $\mu$ g of purified DNA were nebulized by high pressure treatment with argon gas in a reaction chamber for 150 seconds. The ends of the sheared and reprecipitated DNA were blunt-ended by a T4 DNA polymerase-mediated fill in reaction. DNA fragments in the size range between 800 bp and 3 kb were isolated from agarose  
15 gels using a DNA isolation kit (Jetsorb, Genomed Inc., U.S.A.), subcloned into the pBluescript SK vector (Stratagene) and propagated in *E. coli* DN5 $\alpha$ . Clones carrying BAC F15 derived inserts were selected by hybridization using the sheared DNA of BAC F15 as a probe. Sequencing reactions were performed as  
20 described above. Evaluation of the sequencing data, construction of sequence contigs, and estimation of coding propabilities were done by means of the STADEN software package for Unix users (4th edition, 1994). Assessment of coding probabilities was based on a combined evaluation of uneven  
25 positional base frequencies, positional base preference and barley codon usage in the investigated contigs. Homology searches were done using the BLAST software.

*PCR-based Sequencing of Alleles at Mlo*

Plant chromosomal DNA for this purpose was isolated according to Chunwongse et al. (1993). DNA sequences of *Mlo* alleles of the different barley varieties, *mlo* mutants, BC lines, and intragenic recombinants used in this study were obtained by PCR-based sequencing. Seven overlapping subfragments of the gene (each 400 bp-600 bp in length) were amplified by PCR (35 cycles, 60°C annealing temperature) using sets of specific primers. After preparative agarose gel electrophoresis and isolation of the amplification products using the Jetsorb kit (Genomed Inc., U.S.A.) fragments were reamplified to increase specificity. The resulting products were subsequently purified from nucleotides and oligonucleotides (Jetpure, Genomed Inc., U.S.A.) and used as a template in DNA sequencing reactions (see above). All DNA sequences of mutant alleles and corresponding regions of the parental lines and the intragenic recombinants were derived from both strands and confirmed two times in independent sets of experiments. In addition, mutant alleles *mlo*-1, *mlo*-3, *mlo*-4, *mlo*-5, *mlo*-7, *mlo*-8, *mlo*-9, and *mlo*-10 were also verified in the corresponding BC lines in cultivar Ingrid.

*RT-PCR and Rapid Amplification of cDNA Ends (RACE)*

RT-PCR was performed using the SUPERSCRIPT preamplification system for first strand cDNA synthesis (Gibco BRL). Total RNA (1 µg) of seven-day-old primary barley leaves (cultivar Ingrid) served as template. First strand cDNA

synthesis was primed by an oligo(dT) primer. The putative coding region of the *Mlo* gene was subsequently amplified using oligonucleotides 25L (GTGCATCTGCGTGTGCGTA) and 38 (CAGAAACTTGTCTCATCCCTG) in a single amplification step (35 cycles, 60°C annealing temperature). The resulting product was analyzed by direct sequencing. 5'- and 3'-ends of the *Mlo* cDNA were determined by RACE (Frohman et al., 1988) using the MARATHON cDNA amplification kit (Clontech). Corresponding experimental procedures were mainly carried out according to the instructions of the manufacturer. To obtain specific RACE products, two consecutive rounds of amplification (35 cycles, 55°C annealing temperature) were necessary. For this purpose, two sets of nested primers were used in combination with the adapter primers of the kit: oligonucleotides 46 (AGGGTCAGGATCGCCAC) and 55 (TTGTGGAGGCCGTGTTCC) for the 5'-end and primers 33 (TGCAGCTATATGACCTTCCCCCTC) and 37 (GGACATGCTGATGGCTCAGA) for the 3'-end. RACE products were subcloned into pBluescript SK (Stratagene). Ten 5'-end and eight 3' end clones were chosen for DNA sequence analysis.

The term "AFLPs" is used herein to refer to "AFLP markers".



Table 1 summarizes the identified mutation sites of various mutants within the *Mlo* gene. The origin, the mutagen and the predicted effect of the mutation at the amino acid level are indicated.

5        Table 2 shows the results of heteroallelic *mlo* crosses and selfings of the respective *mlo* lines to isolate intragenic recombination events.

10        Table 3 summarizes the genotypes at flanking RFLP markers in susceptible  $F_2$  or homozygous  $F_3$  progeny from the intermutant crosses. CO and NCO indicate crossover type and non crossover type recombinants deduced from flanking molecular marker exchange. Table 3 summarizes DNA sequence analysis of susceptible intragenic crossover type recombinants (from homozygous susceptible  $F_3$  progeny) and the corresponding  
15        parental *mlo* mutant lines. Sequences flanking the identified mutation sites are shown.

20        Table 4 shows the results of direct PCR sequencing of genomic DNA of susceptible intragenic recombinants derived from both heteroallelic crosses *mlo-1* x *mlo-8* and *mlo-1* x *mlo-5*, revealing restoration of wild type sequences.

Table 5 shows several *Arabidopsis thaliana* and two rice expressed sequence tags (ESTs) with homology to the *Mlo* protein.

25        Table 5A show amino acid sequences, with "query" indicating part of the *Mlo* protein sequence to which homology has been found, with the predicted amino acid sequence of each identified EST marked with "subject".

Table 5B shows EST nucleotide sequences encoding the amino acid sequences shown in Table 5A. GenBank Accession number T22145 (definition 4153 Arabidopsis thaliana cDNA clone 97N8T7, NCBI Seq ID 932185), number T22146 (definition 4153 Arabidopsis thaliana cDNA clone 97N9T7, NCBI Seq ID 932186), number N37544 (definition 18771 Arabidopsis thaliana cDNA clone 205N12T7, NCBI Seq ID 1158686), number T88073 (definition 11769 Arabidopsis thaliana cDNA clone 155I23T7, NCBI Seq ID 935932) number H76041 (definition 17746 Arabidopsis thaliana cDNA clone 193P6T7, NCBI seq ID 1053292), number D24287 (rice cDNA partial sequence R1638\_1A, nID g428139) and D24131 (rice cDNA partial sequence R1408\_1A, nID g427985) are shown. The Arabidopsis sequences are from Newman et al. (1994) *Plant Physiol.* 106 1241-55. The rice sequences are from Minobe, Y. and Sasaki, T. submitted 2 Nov 1993 to DDBJ.

Table 1. *mlo* Mutant Alleles

Allele	Mother Variety	Mutagen	Mutational Event at <i>Mlo</i>	Effect on Amino Acid Level
<i>mlo-1</i>	Haisa	X-rays	T <sup>484</sup> → A	trp <sup>182</sup> → arg
<i>mlo-3</i>	Malteria Heda	γ-rays	deletion of 2 nucleotides (1188-1189)	frame shift after phe <sup>395</sup>
<i>mlo-4</i>	Foma	X-rays	deletion of 11 nucleotides (478-488)	frame shift after trp <sup>159</sup>
<i>mlo-5</i>	Carlsberg II	EMS	G <sup>3</sup> → A	met <sup>1</sup> → ilea
<i>mlo-7</i>	Carlsberg II	EMS	G <sup>677</sup> → A	gly <sup>226</sup> → asp
<i>mlo-8</i>	Carlsberg II	EMS	A <sup>1</sup> → G	met <sup>1</sup> → vala
<i>mlo-9</i>	Diamant	EMS	C <sup>28</sup> → T	arg <sup>10</sup> → trp
<i>mlo-10</i>	Foma	γ-rays	deletion of 6 nucleotides (543-548)	2 amino acids (phe <sup>182</sup> , thr <sup>183</sup> ) missing
<i>mlo-12</i>	Elgina	NMU	C <sup>720</sup> → A	phe <sup>240</sup> → leu
<i>mlo-13</i>	Plena	EMS	T <sup>89</sup> → A	val <sup>30</sup> → glu
<i>mlo-16</i>	Alsa	EMS	G <sup>1917</sup> → A	alteration in 3' splice border of intron 9
<i>mlo-17</i>	Plena	EMS	C <sup>92</sup> → T	ser <sup>31</sup> → phe
<i>mlo-26</i>	Plena	EMS	T <sup>609</sup> → A	leu <sup>270</sup> → his
<i>mlo-28</i>	Nadja	NaN <sub>3</sub>	C <sup>665</sup> → T	thr <sup>222</sup> → ile

Numbers of nucleotides and amino acids are given according to the translational start site of the *Mlo* cDNA sequence.

\* Nucleotide number according to the translational start site of the genomic *Mlo* DNA sequence.

EMS = ethylmethane sulfonate, NMU = nitrosomethylurea, NaN<sub>3</sub> = sodium azide.

a Next start codon is at nucleotide positions 79-81 and is in frame with the coding sequence.

Table 2

F<sub>2</sub> progeny from intermutant crosses and selfings

Testcrosses	resistant	susceptible	frequency of susceptible F <sub>2</sub> progeny
<i>mlo-8</i> x <i>mlo-1</i>	5,281	3	$5,7 \times 10^{-4}$
<i>mlo-5</i> x <i>mlo-1</i>	915	0	---
<i>mlo-5</i> x <i>mlo-1</i>	14,474	9	$6,2 \times 10^{-4}$
selfings	resistant	susceptible	
<i>mlo-1</i>	12,634	0	
<i>mlo-5</i>	5,498	0	
<i>mlo-8</i>	8,435	0	

Table 3. Genotypes at Flanking RFLP Markers in Susceptible Progeny Derived from Heteroallelic *mlo* Crosses

Testcross	Susceptible Plant	Parental Genotype in Centromeric Orientation to <i>Mlo</i> <sup>1</sup>	Parental Genotype in Telomeric Orientation to <i>Mlo</i> <sup>2</sup>	Type of Recombination
<i>mlo-8</i> x <i>mlo-1</i>	1	<i>mlo-1</i>	<i>mlo-8</i>	CO
	2	<i>mlo-1</i>	<i>mlo-8</i>	CO
	3	<i>mlo-8</i>	<i>mlo-8</i>	NCO
<i>mlo-1</i> x <i>mlo-5</i>	1	<i>mlo-1</i>	<i>mlo-5</i>	CO
	2	<i>mlo-1</i>	<i>mlo-5</i>	CO
	3	<i>mlo-5</i>	<i>mlo-5</i>	NCO
	4	<i>mlo-1</i>	<i>mlo-5</i>	CO
	5	<i>mlo-5</i>	<i>mlo-5</i>	NCO
	6	<i>mlo-5</i>	<i>mlo-5</i>	NCO
	7	<i>mlo-1</i>	<i>mlo-5</i>	CO
	8*	<i>mlo-5</i>	<i>mlo-5</i>	NCO
	9*	<i>mlo-1</i> + <i>mlo-5</i>	<i>mlo-5</i>	CO

<sup>1</sup>deduced from alleles of RFLP marker WG114 (see Figure 1)<sup>2</sup>deduced from alleles of RFLP marker ABG366 (see Figure 1)

CO = cross over type, NCO = non cross over type of recombination

\* Genotypes of flanking RFLP markers have been determined in heterozygous susceptible F<sub>2</sub> individuals; in all other cases homozygous susceptible F<sub>3</sub> progeny derived from the susceptible F<sub>2</sub> individuals were tested

Table 4. Restoration of *Mlo* Wild Type Sequences by Intragenic Recombination Events

		Nucleotide Sequences Flanking Mutant Sites <sup>1</sup>	
		Nucleotides -3 to +3	Nucleotides 481 to 486
Haisa	<i>Mlo</i>	CCGATG	AATGGG
Carlsberg II	<i>mlo-1</i>	CCGATG	AAAGGG
	<i>Mlo</i>	CCGATG	AATGGG
	<i>mlo-5</i>	CCGATA	AATGGG
	<i>mlo-8</i>	CCGGTG	AATGGG
	Intragenic recombinant <i>mlo-1</i> x <i>mlo-8</i>	CCGATG	AATGGG
<i>mlo-1</i> x <i>mlo-5</i>		CCGATG	AATGGG
	1	CCGATG	AATGGG
	2	CCGATG	AATGGG
	4	CCGATG	AATGGG
	7	CCGATG	AATGGG
<i>mlo-1</i> x <i>mlo-5</i>		CCGATG	AATGGG
	9	CCGATG	AATGGG

<sup>1</sup> Numbers of nucleotides are given according to the translational start site (see Figure 2).

TABLE 5A

>EM EST1:AT1452 T22145 4153 Arabidopsis thaliana cDNA clone 97N8T7. 11/95  
Length = 382

## Plus Strand HSPs:

Score = 248 (115.9 bits), Expect = 2.9e-27, P = 2.9e-27  
Identities = 47/100 (47%), Positives = 67/100 (67%), Frame = +2

Query: 242 KYIKRSMEDDFKVVVGISLPLWGVAILTLFLDINGVGTLIWISFIPLVILLCVGTKLEMI 301  
KY+ R++EDDFK VVGIS LW ++ L++NG T WI+FIP +LL VGKLE +  
Sbjct: 2 KYMRALEDDEDFKQVVGISWYLWXFVVFIXLLNVNGWHTYFWIAFIPFXLLAVGKLEHV 181

Query: 302 IMEMALEIQDRASVIKGA PVVEPSNKFFWFH RPDWVLF 341  
I ++A E+ ++ I+G VV+P . + FWF +P VL+ I  
Sbjct: 182 IAQLAHEVAEKHVAIEGDLVVKPXXEHFWFSKPQIVLYLI 301

>EM EST1:AT1462 T22146 4154 Arabidopsis thaliana cDNA clone 97N9T7. 11/95  
Length = 390

## Plus Strand HSPs:

Score = 212 (99.1 bits), Expect = 4.2e-26, Sum P(2) = 4.2e-26  
Identities = 41/83 (49%), Positives = 58/83 (69%), Frame = +2

Query: 242 KYIKRSMEDDFKVVVGISLPLWGVAILTLFLDINGVGTLIWISFIPLVILLCVGTKLEMI 301  
KY+ R++EDDFK VVGIS LW ++ L L++NG T WI+FIP +LL VGKLE +  
Sbjct: 2 KYMRALEDDEDFKQVVGISWYLWXFVVFIFLLNVNGWHTYFWIAFIPFALLAVGKLEHV 181

Query: 302 IMEMALEIQDRASVIKGA PVVEP 324  
I ++A E+ ++ I+G VV+P  
Sbjct: 182 IAQLAHEVAEKHVAIEGDLVVKP 250

Score = 52 (24.3 bits), Expect = 1.9, Sum P(2) = 0.85  
Identities = 9/32 (28%), Positives = 16/32 (50%), Frame = +2

Query: 18 WAVAVVFAAMVLVSVIMEHGLHKLGHWFQHRH 49  
W + FA ++ V +EH + +L H +H  
Sbjct: 122 WIAFIPFALLAVGKLEHVIAQLAHEVAEKH 217

Score = 49 (22.9 bits), Expect = 4.2e-26, Sum P(2) = 4.2e-26  
Identities = 8/17 (47%), Positives = 12/17 (70%), Frame = +1

Query: 323 EPSNKFFWFH RPDWVLF 339  
E S++ FWF +P VL+  
Sbjct: 244 ETSDEHFWFSKPQXVLY 294

TABLE 5A cont'd

>EM EST1:AT54418 N37544 18771 Arabidopsis thaliana cDNA clone 205N12T7. 1/96  
Length = 585

## Plus Strand HSPs:

Score = 277 (129.5 bits), Expect = 1.2e-45, Sum P(2) = 1.2e-45  
Identities = 51/96 (53%), Positives = 71/96 (73%), Frame = +1

Query: 236 SKFDFHKKYIKRSMEDDFKVVVGISLPLWGVAILTLFLDINGVGTLIWISFIPLVILLCVG 295  
S+EDF KYI+RS+E DFK VV.IS +W VA+L L + G+ + +W+ FIPLV++L VG  
Sbjct: 127 SRFD FRKYIQRSLKDFKTVVEISPVIWFAVLFLLTNSYGLRSYLWLPFIPLVVILIVG 306

Query: 296 TKLEMIIMEMALEIQDRASVIKAPVVEPSNKF FWF 331  
TKLE+II ++ L IQ+ V++GAPVV+P + FWF  
Sbjct: 307 TKLEVIITKLGLRIQEEDVVRGAPVVQPGDDXFWF 414

Score = 121 (56.6 bits), Expect = 1.2e-45, Sum P(2) = 1.2e-45  
Identities = 25/45 (55%), Positives = 29/45 (64%), Frame = +1

Query: 196 SSTPGIRWVVAFFRQFFRSVTKVDYLT LRAGFINAHLSONSKFDF 240  
S T W+V FFRQFF SVTKVDYL L GFI AH + ++ F  
Sbjct: 1 SKTRVTLWIVCFRQFFGSVTKVDYLALXHGFI MAHFAPGNESRF 135

>EM EST1:AT04117 H76041 17746 Arabidopsis thaliana cDNA clone 193P6T7. 11/95  
Length = 476

## Plus Strand HSPs:

Score = 210 (98.2 bits), Expect = 9.0e-36, Sum P(2) = 9.0e-36  
Identities = 43/86 (50%), Positives = 58/86 (67%), Frame = +1

Query: 196 SSTPGIRWVVAFFRQFFRSVTKVDYLT LRAGFINAHLSONSKFDFHKKYIKRSMEDDFKVV 255  
++TP V FFRQFF SV + DYLT LR GF +AHL+ KF+F +YIK S+EDDFK+V  
Sbjct: 124 TTPFXFNVGCFRQFFVSVERTDYLT LRHGFXSAHLAPGRKFNFQRYIKXSLEDDFKLV 303

Query: 256 VGISLPLWGVAILTLFLDINGVGT LI 281  
VGI LW ++ L + +GT++  
Sbjct: 304 VGIXPVLWASFVIFLAVQX\*WLG TIV 381

Score = 119 (55.6 bits), Expect = 9.0e-36, Sum P(2) = 9.0e-36  
Identities = 24/57 (42%), Positives = 32/57 (56%), Frame = +1

Query: 156 MRTWKKWETETTSLEYQFANDPARFRFTHQTSFVKRHLGLSSTPGIRWVVAFFRQFF 212  
+R WKKWE T S +Y F D +R R TH+TSFV+ H +T + V F + F  
Sbjct: 1 IRGWKKWEQXTLSNDYXFXIDHSRLRLTHETSFVREHTSFWTTPFXFNVGCFRQF 171

Score = 40 (18.7 bits), Expect = 1.2e-08, Sum P(2) = 1.2e-08  
Identities = 8/19 (42%), Positives = 10/19 (52%), Frame = +2

Query: 269 TLFLDINGVGT LIWISFIP 287  
+L + NG G L W S P  
Sbjct: 344 SLLFNXNGWGPLFWASVPP 400



99

TABLE 5A cont'd

>EM EST1:AT0739 T88073 11769 Arabidopsis thaliana cDNA clone 155I23T7. 11/95  
Length = 460

Plus Strand HSPs:

Score = 175 (81.8 bits), Expect = 1.2e-24, Sum P(2) = 1.2e-24  
Identities = 31/67 (46%), Positives = 43/67 (64%), Frame = +1

Query: 146 VITIALSRLKMRTWKKWETETTSLEYQFANDPARFRFTHQTSFVKRHLGLSSTPGIRWVV 205  
++T A ++KMRTWK WE ET ++EYQ++NDP RFRF TSF +RHL S + +  
Sbjct: 4 IVTYAFGKIKMRTWKSWEETKTIEYQYSNDPERFRFARDTSFGRRHLNFWWSKTRVTLWI 183

Score = 121 (56.6 bits), Expect = 1.4e-14, Sum P(2) = 1.4e-14  
Identities = 25/45 (55%), Positives = 29/45 (64%), Frame = +1

Query: 196 SSTPGIRWVVAFFRQFFRSVTKVDYLTLRAGFINAHLSONSKFDF 240  
S T W+V FFRQFF SVTKVDYL L GFI AH + ++ F  
Sbjct: 157 SKTRVTLWIVCFRQFFGVS TKVDYLALXHGFI MAHFAPGNESRF 291

Score = 75 (35.1 bits), Expect = 1.2e-24, Sum P(2) = 1.2e-24  
Identities = 14/21 (66%), Positives = 17/21 (80%), Frame = +1

Query: 236 SKFDFHKYIKRSMEDDFKVVV 256  
S+FDF KYI+RS+ DFK VV  
Sbjct: 283 SRFDFRKYIQRSLXDFKTVV 345

>EM EST5:OSR16381A D24287 Rice cDNA, partial sequence (R1638\_1A). 5/95  
Length = 400  
Plus Strand HSPs:

Score = 147 (68.7 bits), Expect = 1.9e-16, Sum P(2) = 1.9e-16  
Identities = 26/53 (49%), Positives = 35/53 (66%), Frame = +1

Query: 236 SKFDFHKYIKRSMEDDFKVVVGISLPLWGVAILTLFLDINGVGTLIWISFIPL 288  
++F+F KYIKR +EDDFK VVGIS P W A+ + +++G L W S PL  
Sbjct: 202 TRFNFRKYIKRXLEDDFKTVVGISAPXWASALAIMLNFVHGWNNLFWFSTXPL 360

Score = 45 (21.0 bits), Expect = 1.9e-16, Sum P(2) = 1.9e-16  
Identities = 9/15 (60%), Positives = 11/15 (73%), Frame = +2

Query: 287 PLVILLCVGKLEMI 301  
PL + L VGTKL+ I  
Sbjct: 356 PLXVTLAVGKTLQAI 400

>EM EST5:OSS1692A D39989 Rice cDNA, partial sequence (S1692\_1A). 11/94  
Length = 343

Plus Strand HSPs:

Score = 95 (44.4 bits), Expect = 0.00059, P = 0.00059  
Identities = 24/58 (41%), Positives = 31/58 (53%), Frame = +3

Query: 43 HWFQHRHKKALWEALEKMKAEMLVGFISLLLVTDPIIAKICISEDAAVMWPCKR 100  
H + H+ L +A+EKMK E+ML+GFISLL T I S+ PC R  
Sbjct: 3 HXSEKTHRNPLHKAMEKMK EEMMLLGFISLLLAATSRIISGICIDSKYYNSNFSPCTR 176

TABLE 5B

100

GenBank Accession Number T22145

```

1  caagtatatg atgcgcgctc tagaggatga tttcaaacaa gttgttggtg ttagttggta
61  tcttttgntc tttgtcgta tcttttttct gctaaatgtt aacggatggc acacatattt
121 ctggatagca tttattccct ttcttttctg tcttgctgtg ggaacaaagt tggagcatgt
181 nattgcacag ttagctcatg aagttgcaga gaaacatgta gccattgaag gagacttagt
241 ggtgaaaccc ncanatgagc atttctgggt cagcaaacct caaattgttc tctacttgat
301 cccattttat cctctttccc agaatgcntt ttnagantgc nttttttntt tttggnnntt
361 ggggtaanan annggtttcg nc

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GenBank Accession Number T22146

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1  caagtatatg atgcgcgctc tagaggatga tttcaaacaa gttgttggtg ttagttggta
61  tcttttgntc tttgtcgta tcttttttct gctaaatgtt aacggatggc acacatattt
121 ctggatagca tttattccct ttgctttgct tcttgctgtg ggaacaaagt tggagcatgt
181 nattgcacag ttagctcatg aagttgcaga gaaacatgta gccattgaag gagacttagt
241 ggtgaaacct cagatgagca tttctgggtc agcaaacctc aaantgttct ctactngatc
301 cncctttatcc cccttccaga atgccttttt nangattcnn ntttttctct nttgganntt
361 ttgggnnnnc aaacgggntt nggacctccg

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GenBank Accession Number N37544

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1  agcaagacga gagtcacact atggattgtt tgttttttta gacagttctt tggatctgtc
61  accaaagtgt attacttagc actaagnat ggtttcatca tggcgcatth tgctcccggt
121 aacgaatcaa gattcgattt ccgcaagtat attcagagat cattagagaa agacttcaaa
181 accgttggtg aaatcagtcg gggtatctgg tttgtcgctg tgctattcct cttgaccaat
241 tcatatggat tacgtttotta cctctgggtt ccattcattc cactagtcgt aattctaata
301 gttggaacaa agcttgaagt cataataaca aaattgggtc taaggatcca agaggaaggt
361 gatgtgggtg gaggcgcccc agtggttcag cctggtgatg accncttctg gtttngnaan
421 cacgnntcaa tnttttccnt antcacttng gcctttttan ggggtgaattt caacttcatt
481 ctttncctgg ggnccgatga ttcaatccaa naatnttccc ctgaagnctn caagtttggg
541 cataggcttt nggtgggntt ttcaganttt nagtttggct tcccc

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TABLE 5B (Continued)

## GenBank Accession Number T88073

1 tgcattgtta cttatgcttt cggaagatc aagatgagga cgtggaagtc gtgggaggaa  
 61 gagacaaaga caatagagta tcagtattcc aacgatcctg agagggttcag gtttgcnaagg  
 121 gacacatctt ttgggagaag acatctcaat ttctggagca agacgagagt cacactatgg  
 181 attgtttgtt tttttagaca gttcttttga tctgtcacca aagttgatta cttagcacta  
 241 agncatgggt tcatcatggc gcatttttgc cccggtaacg aatcaagatt cgatttccgc  
 301 aagtatatcc agagatcatt agngnaagac ttcaaaaccg ttgtttgaaa tcagtccggg  
 361 tatctgggtt gtcggctgtg ctattccnct tgaccaattc atatggntnc ggtnttncnc  
 421 tggtagcatt attcnctagc ggaatntaaa agttggcnga

## GenBank Accession Number H76041

1 attcgtggat ggaaaaagtg ggagcaagan acattatcta atgactatna gtttntctatt  
 61 gatcattcaa gacttaggct cactcatgag acttcttttg tnagagaaca tacaagtttc  
 121 tggacaacaa cncctttctn ctttaacgct ggatgcttct ttaggcagtt ctttgtatct  
 181 gtngaaagaa cggactactt gactctgccc catggattca nctctgccc ttttagctcca  
 241 ggaagaaagt tcaacttcca gagatatatc aaangatttc tcgaggatga tttcaagttg  
 301 gtagttggaa taagnccagt tctttgggca tcatttgtaa tcttccttgc tgttcaatgn  
 361 taatggctgg ggaccattgt tttgggcntc ggtaccgcct ntactcanaa ncccaggctt  
 421 ttggccaagg ttcaaggaat ttngggacaa tggggtagaa tcgtgggcnc atnngg

## GenBank Accession Number D24287

1 tcntntttnn ttttcgnntn cntccacccc tnnntnctc nancncnttn nnttatctc  
 61 tntntntntc ncntntccn ncaccacnn ncgacgggcn tggactnngc ccnnngttcg  
 121 aggctgccc ctgncgtctg agacctacct tgncaattga cggcacngga cttcanttgc  
 181 tgcactctt atctctacgg gactagggtc aattttcgga aatacatcaa aaggncactg  
 241 gaggacgatt ttaagacagt tgttggcatt agtgacccn tatgggcttc tgcgttggcc  
 301 attatgctct tcaatgttca tggatggcat aacttgttct ggttctctac aatnccctt  
 361 gntagtaact ttagcagttg gaacaaagct gcaggctata

## GenBank Accession Number D24131

1 cagactacct gactttgagg cacggattca ttgctgctca tttatctcta gggactagggt  
 61 tcaattttcg gaaatacatc aaaagggtcac tggaggacga ttttaagaca gttgttggca  
 121 ttagtgacc cttatgggct tctgcgttgg ccattatgct cttnaatgtt catggatggc  
 181 ataacttgtt ctggttctct acaatcccc ttgtagtaac ttttagcagtt ggaacaaagc  
 241 tgcaggctat aattgcaatg atggctgttg aaattaaaga gaggcataca gtaattcaag  
 301 gaatgccggt ggtgaactca gtgat

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CLAIMS:

1. An isolated polynucleotide encoding a polypeptide which includes the amino acid sequence shown in Figure 2.

5

2. A polynucleotide according to claim 1 wherein the coding sequence is the coding sequence shown in Figure 2.

3. A polynucleotide according to claim 1 wherein the coding  
10 sequence is a mutant, allele, variant or derivative of the coding sequence shown in Figure 2, by way of addition, deletion, substitution and/or insertion of one or more nucleotides.

15 4. An isolated polynucleotide which on expression in a transgenic plant exerts a negative regulatory effect on a pathogen defence response of the plant, which defence response is pathogen independent and autonomous of the presence of pathogen, the polynucleotide encoding a polypeptide which  
20 includes an amino acid sequence which is a mutant, allele, variant or derivative of the Barley Mlo sequence shown in Figure 2, or is a homologue of another species or a mutant, allele, variant or derivative thereof, the amino acid sequence differing from that shown in Figure 2 by way of addition,  
25 substitution, deletion and/or insertion of one or more amino acids.

5. A polynucleotide according to claim 4 encoding a polypeptide which includes the amino acid sequence shown in Figure 13.

5 6. A polynucleotide according to claim 5 wherein the coding sequence is that shown in Figure 10.

7. A polynucleotide according to claim 5 wherein the coding sequence is a mutant, allele, variant or derivative of the  
10 coding sequence shown in Figure 10, by way of addition, deletion, substitution and/or insertion of one or more nucleotides.

8. A polynucleotide according to claim 4 encoding a  
15 polypeptide which includes the amino acid sequence shown in Figure 14.

9. A polynucleotide according to claim 8 wherein the coding sequence is that shown in Figure 11.

20

10. A polynucleotide according to claim 8 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence shown in Figure 11, by way of addition, deletion, substitution and/or insertion of one or more  
25 nucleotides.

11. A polynucleotide according to claim 4 encoding a

polypeptide which includes the amino acid sequence shown in Figure 15.

12. A polynucleotide according to claim 11 wherein the coding  
5 sequence is that shown in Figure 12.

13. A polynucleotide according to claim 11 wherein the coding  
sequence is a mutant, allele, variant or derivative of the  
coding sequence shown in Figure 12, by way of addition,  
10 deletion, substitution and/or insertion of one or more  
nucleotides.

14. A polynucleotide according to any preceding claim operably  
linked to a regulatory sequence for expression.  
15

15. An isolated polynucleotide encoding a polypeptide which on  
expression in a transgenic plant produces a polypeptide which  
can stimulate or maintain a defence response of the plant, the  
encoded polypeptide including an amino acid sequence which is a  
20 mutant, allele, variant or derivative of the Barley *Mlo*  
sequence shown in Figure 2 or of a homologue of another  
species, the amino acid sequence differing from that shown in  
Figure 2 by way of addition, substitution, deletion and/or  
insertion of one or more amino acids.

25

16. A polynucleotide according to claim 15 which stimulates or  
maintains said defence response of the plant on homozygous

expression in the plant.

17. A polynucleotide according to claim 15 wherein the amino acid sequence includes an alteration identified in Table 1.

5

18. A polynucleotide according to claim 17 wherein the amino acid sequence is that of Figure 2 including a substitution at residue 240.

10

19. A polynucleotide according to claim 17 wherein the amino acid sequence includes Leucine at residue 240.

20. A polynucleotide according to any of claims 15 to 19 operably linked to a regulatory sequence for expression.

15

21. An isolated polynucleotide which has at least about 600 contiguous nucleotides of the nucleotide sequence of any of claims 1 to 13 or complement thereof

20

22. A polynucleotide according to claim 21 operably linked to a regulatory sequence for transcription.

25

23. An isolated polynucleotide which has at least about 300 contiguous nucleotides of the sequence of any of claims 1 to 13, or complement thereof, operably linked to a regulatory sequence for transcription.

24. A polynucleotide according to claim 22 or claim 23 wherein the regulatory sequence includes an inducible promoter.

25. A nucleic acid vector suitable for transformation of a host cell and including a polynucleotide according to any preceding claim.

26. A nucleic acid vector according to claim 25 wherein said host cell is a microbial cell.

27. A nucleic acid vector according to claim 25 wherein said host cell is a plant cell.

28. A host cell containing a heterologous polynucleotide or nucleic acid vector according to any preceding claim.

29. A cell according to claim 28 which is microbial.

30. A cell according to claim 28 which is a plant cell.

31. A cell according to claim 30 having said heterologous polynucleotide incorporated within its genome.

32. A cell according to claim 31 having more than one said polynucleotide per haploid genome.

33. A cell according to any of claims 30 to 32 which is

comprised in a plant.

34. A plant including a cell according to any of claims 30 to 32.

5

35. A plant which is a sexually or asexually propagated off-spring, clone or descendant of a plant according to claim 34, or any part or propagule of said plant, off-spring, clone or descendant.

10

36. A part or propagule of a plant according to claim 35.

37. A plant according to claim 34 which does not breed true.

15 38. A method of producing a plant, the method including incorporating a heterologous polynucleotide according to any of claims 1 to 14 into a plant cell and regenerating a plant from said plant cell.

20 39. A method of producing a plant, the method including incorporating a heterologous polynucleotide according to any of claims 15 to 20 into a plant cell and regenerating a plant from said plant cell.

25 40. A method of producing a plant, the method including incorporating a heterologous polynucleotide according to any of claims 21 to 24 into a plant cell and regenerating a plant from

said plant cell.

41. A method according to any of claims 38 to 40 including sexually or asexually propagating or growing off-spring or a descendant of said plant.

42. A method of stimulating a defence response in a plant, the method including causing or allowing transcription from a heterologous polynucleotide according to any of claims 1 to 14 within cells of the plant.

43. A method of stimulating a defence response in a plant, the method including causing or allowing transcription from a heterologous polynucleotide according to any of claims 15 to 20 within cells of the plant.

44. A method of stimulating a defence response in a plant, the method including causing or allowing transcription from a heterologous polynucleotide according to any of claims 21 to 24 within cells of the plant.

45. A method of producing a polynucleotide encoding a polypeptide which on expression in a transgenic plant produces a polypeptide which can stimulate or maintain a defence response of the plant, the method including alteration of the nucleotide sequence of a polynucleotide according to any of claims 1 to 14.



46. A method according to claim 45 involving site-specific sequence mutation.

47. A method according to claim 45 involving intracellular  
5 homologous recombination.

48. A method wherein following alteration of a nucleotide sequence in accordance with the method of claim 45 a polynucleotide including the altered nucleotide sequence is  
10 introduced into a host cell.

49. A method according to claim 48 wherein the host cell is a plant cell.

15 50. A method wherein following introduction of a polynucleotide into a plant cell in accordance with claim 49 a plant is regenerated from the cell or descendants thereof including the altered nucleotide sequence.

20 51. Use of a polynucleotide according to any of claims 1 to 14 for stimulating a defence response in a plant.

52. Use of a polynucleotide according to any of claims 15 to  
20 for stimulating a defence response in a plant.

25

53. Use of a polynucleotide according to any of claims 21 to  
24 for stimulating a defence response in a plant.

54. Use of a polynucleotide according to any of claims 21 to 24 for down-regulation of expression of a gene encoded a polypeptide encoded by a polynucleotide according to any of claims 1 to 14.

5

55. Use of a polynucleotide according to any of claims 1 to 14 in the production of a transgenic plant.

56. Use of a polynucleotide according to any of claims 15 to 20 in the production of a transgenic plant.

57. Use of a polynucleotide according to any of claims 21 to 24 in the production of a transgenic plant.

58. A method of determining the presence of a pathogen resistance or susceptibility allele in a plant or plant cell, the method including analysing a sample from the plant or plant cell by:

(a) comparing the sequence of nucleic acid in the sample with all or part of the nucleotide sequence shown in Figure 7 to determine whether the sample from the patient contains a mutation;

(b) determining the presence in the sample of a polypeptide including the amino acid sequence shown in Figure 7 or a fragment thereof and, if present, determining whether the polypeptide is full length, and/or is mutated, and/or is expressed at the normal level;

(c) performing DNA fingerprinting to compare the restriction pattern produced when a restriction enzyme cuts nucleic acid in the sample with the restriction pattern obtained from the nucleotide sequence shown in Figure 7 or from  
5 a known mutant, allele or variant thereof;

(d) contacting the sample with a specific binding member capable of binding to nucleic acid including the nucleotide sequence as set out in Figure 7 or a fragment thereof, or a mutant, allele or variant thereof, the specific binding member  
10 including nucleic acid hybridisable with the sequence of Figure 7 or a polypeptide including a binding domain with specificity for nucleic acid including the sequence of Figure 7 or the polypeptide encoded by it, or a mutated form thereof, and determining binding of the specific binding member;

15 (e) performing PCR involving one or more primers based on the nucleotide sequence shown in Figure 7 to screen the sample for nucleic acid including the nucleotide sequence of Figure 7 or a mutant, allele or variant thereof.

20 59. A method of determining the presence of target nucleic acid in a plant or plant cell, the method including contacting a nucleic acid molecule which includes the nucleotide sequence shown in Figure 7 or an oligonucleotide fragment thereof with nucleic acid in a sample from the plant or plant cell and  
25 assessing hybridisation of said nucleic acid molecule with nucleic acid in the sample.

60. A method according to claim 59 which involves amplification of nucleic acid to which said nucleic acid molecule hybridises.

5 61. A method according to claim 59 or claim 60 wherein said nucleic acid molecule includes an alteration in sequence compared with the nucleotide sequence shown in Figure 7 or corresponding fragment thereof.

10 62. A method according to claim 61 wherein said alteration is selected from those shown in Table 1.

63. An assay method for identifying a compound able to bind the polypeptide encoded by the polynucleotide of any of claims  
15 1 to 14 or any of claims 15 to 20, the method including:

- (a) bringing into contact said polypeptide or a fragment thereof, and a test compound; and
- (b) determining interaction or binding between said polypeptide or fragment thereof and the test compound.

20

64. An assay method according to claim 63 wherein a compound is identified which is able to bind the polypeptide for which the amino acid sequence is shown in Figure 2.

25 65. An assay method for identifying a compound able to stimulate a defence response in a plant by interaction with the polypeptide encoded by the polynucleotide of any of claims 1 to

14 or any of claims 15 to 20, the method including:

(a) contacting a plant or plant part with a test compound and determining stimulation of a defence response; and

(b) bringing into contact said polypeptide or a fragment  
5 thereof with a test compound and determining interaction or binding between said polypeptide or a fragment thereof and the test compound;

step (b) being performed with a test compound which tests positive in step (a), or step (a) being performed with a test  
10 compound which tests positive in step (b), or steps (a) and (b) being performed in parallel.

66. An assay method according to claim 65 wherein stimulation of a defence response is determined by monitoring pathogen  
15 growth and/or viability on the plant or plant part.

67. An assay method according to claim 65 or claim 66 wherein a compound is identified which is able to bind the polypeptide for which the amino acid sequence is shown in Figure 2.

20

68. An assay method according to any of claims 65 to 67 wherein a compound is identified which is able to stimulate resistance to powdery mildew in barley.

25 69. A method which includes following identification of a compound as being able to stimulate a defence response in a plant in accordance with any of claims 65 to 68 formulation of

the compound, or optionally if the compound is peptidyl nucleic acid encoding it, into a composition including at least one additional component.

5 70. A method which includes following identification of a compound as being able to stimulate a defence response in a plant in accordance with any of claims 56 to 58 application of the compound, or optionally if the compound is peptidyl nucleic acid encoding it, to a plant.

10

71. Use of a polypeptide encoded by a polynucleotide according to any of claims 1 to 14, in screening for compounds able to stimulate a defence response in a plant.

15 72. Use of a polypeptide encoded by a polynucleotide according to any of claims 15 to 20, in screening for compounds able to stimulate a defence response in a plant.

73. A compound able to stimulate a defence response in a plant  
20 identified by a method according to any of claims 63 to 68.

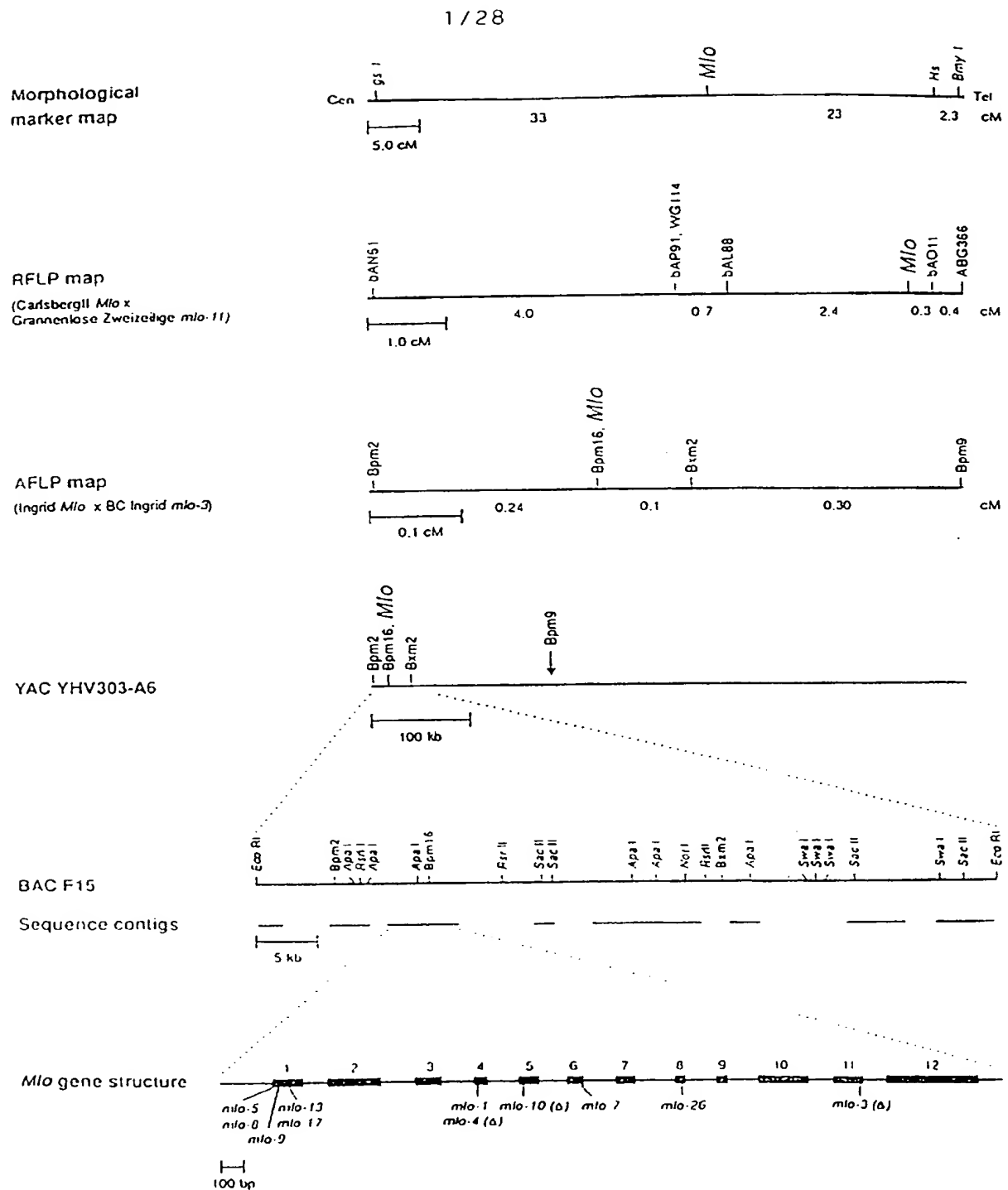


Figure 1

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M S D K K G V P A R E L P E T P S W A V  
 ATGTCGGACAAAAAGGGGTGCCGGCGCGGGAGCTGCCGGAGACGCCGTCGTGGGCGGTG 60  
 A V V F A A M V L V S V L M E H G L H K  
 CGGGTGGTCTTCGCCGCCATGGTGCTCGTGTCCGTCCTCATGGAACACGGCCTCCACAAG 120  
 L G H W F Q H R H K K A L W E A L E K M  
 CTCGGCCATTGGTTCCAGCACCGGCACAAGAAGGCCCTGTGGGAGGCGCTGGAGAAGATG 180  
 K A E L M L V G F I S L L L I V T Q D P  
 AAGGCGGAGCTCATGCTGGTGGGCTTCATATCCCTGCTCCTCATCGTCACGCAGGACCCC 240  
 I I A K I C I S E D A A D V M W P C K R  
 ATCATGCCCAAGATATGCATCTCCGAGGATGCCGCCGACGTCATGTGGCCCTGCAAGCGC 300  
 G T E G R K P S K Y V D Y C P E G K V A  
 GGCACCGAGGGCCGCAAGCCCAGCAAGTACGTTGACTACTGCCCGGAGGGCAAGGTGGCG 360  
 L M S T G S L H Q L H V F I F V L A V F  
 CTCATGTCCACGGGCAGCTTGACACAGCTGCACGTCCTTCATCTTCTGTGCTCGCGGTCTTC 420  
 H V T Y S V I T I A L S R L K M R T W K  
 CATGTCACCTACAGCGTCATCACCATAGCTCTAAGCCGTCCTCAAAATGAGAACATGGAAG 480  
 K W E T E T T S L E Y Q F A N D P A R F  
 AAATGGGAGACAGAGACCACCTCCTTGAATACCAGTTGCGMAATGATCCTGCACGGTTC 540  
 R F T H Q T S F V K R H L G L S S T P G  
 CGGTTACGCACACAGACGTCGTTCCGTGAAGCGCCACCTGGGCCTCTCCAGCACCCCTGGC 600  
 I R W V V A F E R Q F F R S V T K V D Y  
 ATCAGATGGGTGGTGGCCTTCTTCAGGCAGTTCTTCAGGTACAGTCACCAAGGTGGACTAC 660  
 L T L R A G F I N A H L S Q N S K F D F  
 CTGACCTTGAGGGCAGGCTTCATCAACGCGCATTTGTGCGAAAACAGCAAGTTCGACTTC 720  
 H K Y I K R S M E D D F K V V V G I S L  
 CACAAGTACATCAAGAGGTGATGGAGGACGACTTCAAGGTGCTGCTCGGCATCAGCCTC 780  
 P L W G V A I L T L F L D I N G V G T L  
 CCGCTGTGGGGTGTGGCGATCCTCACCTCTTCCTTGACATCAATGGGGTTGGCAGCTC 840  
 I W I S F I P L V I L L C V G T K L E M  
 ATCTGGATTCTTTTCATCCCTCTCGTGATCCTCTTGTGTGTTGGAACCAAGCTGGAGATG 900  
 I I M E M A L E I Q D R A S V I K G A P  
 ATCATCATGGAGATGGCCCTGGAGATCCAGGACCGGGCGAGCGTCATCAAGGGGGCCCC 960  
 V V E P S N K F F W F H R P D W V L F F  
 GTGGTCGAGCCCAAGCAAGTTCTTCTGGTTCCACCGCCCCGACTGGGTCTCTTCTTC 1020  
 I H L T L F Q N A F Q M A H F V W T V A  
 ATACACCTGACGTTGTTCCAGAACGCTTTCAGATGGCGCATTTTGTGTGGACAGTGGCC 1080  
 T P G L K K C Y H T Q I G L S I M K V V  
 ACGCCCGGCTTGAAGAAATGCTACCACACGCAGATCGGGCTGAGCATCATGAAGGTGGTG 1140  
 V G L A L Q F L C S Y M T F P L Y A L V  
 GTGGGGCTAGCTCTCCAGTTCCTCTGCAGCTATATGACCTTCCCCCTCTACGCGCTCGTC 1200  
 T Q M G S N M K R S I F D E Q T S K A L  
 ACACAGATGGGATCAACATGAAGAGGTCCATCTTCGACGAGCAGACGTCCAAGGCGCTC 1260  
 T N W R N T A K E K K K V R D T D M L M  
 ACCAACTGGCGGAACACGGCCAAGGAGAAGAAGAAAGTCCGAGACACGGACATGCTGATG 1320  
 A Q M I G D A T P S R G S S P M P S R G  
 GCTCAGATGATCGGCGACGCAACACCGAGCCGAGGCTCGTCGCCGATGCCGAGCCGGGGC 1380

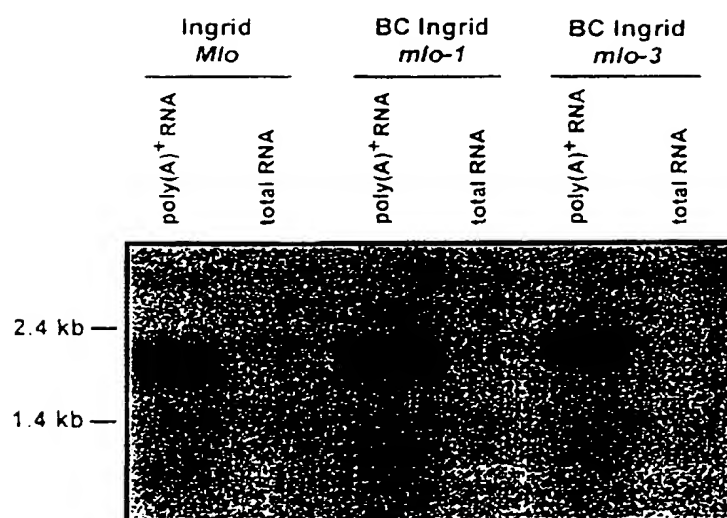
Figure 2



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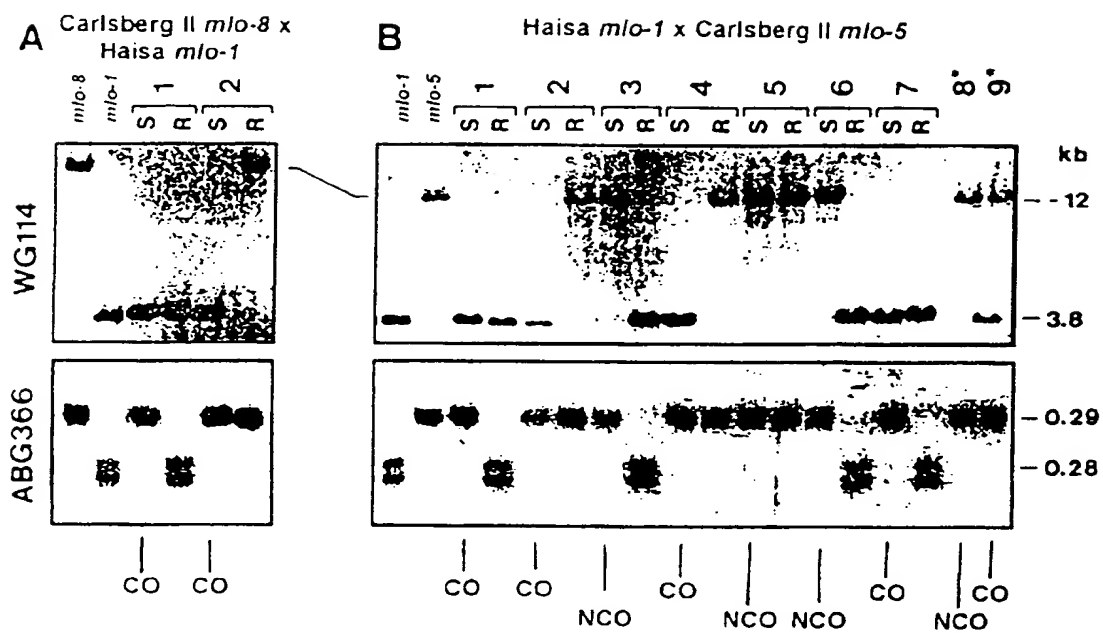
Figure 2 (Continued)

S S P V H L L H K G M G R S D D P Q S A	
TCATCACCCGTGCACCTGCTTCACAAGGGCATGGGGCGGTCGGACGACCCCCAGAGCGCG	1440
P T S P R T Q Q E A R D M Y P V V V A H	
CCCACCTCGCCAAGGACCCAGCAGGAGGCTAGGGACATGTACCCGGTTGTGGTGGCGCAC	1500
P V H R L N P N D R R R S A S S S A L E	
CCGGTGCACAGACTAAATCCTAACGACAGGAGGAGGTCCGCCTCGTCGTCGGCCCTCGAA	1560
A D I P S A D F S F S Q G *	
GCCGACATCCCCAGTGCAGATTTTTCCTTCAGCCAGGGATGA	1602

Figure 3

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Figure 4



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**FIGURE 5**

292 GCGGAGAGCTCATGCTGGGTGGGCTTCATATCCCTGCTCCTCATCGTCACGCA 341  
80 GCANAGCTGATGCTGCTGGGCTTCATNTCCCTGCTTCTCACCCTGGGCACA 129  
342 GGACCCCATCATCGCCCAAGATATGCATCTCCGAGGATGCCGCCGACGTCA 391  
130 GCGGCC...CATCTCCAANATCTGCATCCCCAAGTCGGCTGCCAACATCT 176  
392 TGTGGCCCTGCAAGCGCGGCACCGAGGGCCGC.AAGCCCAGCAAGTACGT 440  
177 TGTGCGCTGCAAGGCAGGCCNAGATGCCATCGAAGAANAAGCAGCAAGT 226  
441 TGACTACTGCCCGGAGGTGAGCAGCAGAGCCCGACCAGCAGCTTCACGA 490  
227 GGTCNCCNGTCC.TTGGCCGGCGCCGGCGGGGACTACTGCTCNAAT 275  
491 TGATGAAGAAATCAATACC.....GAACTTTTTCTTGTTTTCT 528  
276 TCGATGTGAGAATAACNCCAGCTGCCGGCAAGCACAACCTCGATNCNATN 325  
529 TCTGATTGTCGCTTTGGCTTGGCTTAATTGGTGTGTGTGTGTGTGTTGC 578  
326 ACTNATT.....TAACTATAATTGATTTTTCTTGGGTTTTCTGC 364  
579 AGGGCAAGGTGGCGCTCATGTCCACGGGCAGCTTGCACCAGCTGCACGTC 628  
365 AGGGCAAGGTGGCGCTGATGTCGGCAAAGAGCATGCACCAGCTGCACATT 414  
629 TTCATCTTCGTGCTCGCGGTCTTCCATGTACCTACAGCGTCATCACCAT 678  
415 TTCATCTTCGTGCTCGCCGTGTTCCATGTTACCTACTGCATCATCACCAT 464  
579 AGCTCTAAGCCGTCTCAAAGTGAGCCTTTGCTTCT....TCTTCTTCTT 723  
465 GGGTTTAGGGCGCTCAAAGTGAGTTTGTCGTTCTGTCCCTCATGCACAT 514  
724 CTTTTACC.....GCACGTCTGTCTGTACGGCTACCTACCTGTTCA 765  
515 GTTTTCTCTAGTTCTAGCAANATTGTCAGTCCTTCAAATGGATTGTTTCG 564  
766 TCAGGCTTGAGTAAACTGTTCCATAATCTGC.....TCCGGCATAA 807  
565 ACA.....AGAAACCCAATTTATTAATTTGCCAGTTAAATATATAATAA 608  
808 TCCTCTCCTCTG....CAGATGAGAACATGGAAGAAATGGGAGACAGAG 853  
609 TTGATCTTTCTTGGTTTTAGATGAAGAAATGGAAGAAGTGGGAGTCACAG 658  
854 ACCACCTCCTTGGAATACCAGTTTCGCAATGGTCAGGATCCCCACTCTG 903  
659 ACCAACTCATTGGAGTATCAGTTTCGCAATCGGTAGTG.....AATTAA 701  
904 CAATCTCCC...CTTCTTCGAAACCAAACC...TGATGATCCATTTAAA 946  
702 GAATCTCCCTAACTATTTCAATTCAGAACCTTTATGATAATGTCTTGAAA 751  
947 GACGCAGGCACGATCAGAGTGAGTGAAGTATGTATGTTCAATTTTTTGTG 996  
752 GAGGAGGAGCAAAATCAG.CTGAAAAATATGATCGA..... 785  
997 TCCTTTTCAGATCCTGACCGGTTCCGGTTCACGCACCAGACGTCGTTTCGTG 1046  
786 TCCATGCAGATCCTTCACGATTTCAGGTTTCACGCATCAGACGTCGTTTCGTG 835

FIGURE 5 cont'd

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1047 AAGCGCCACCTGGG...CCTCTCCAGCACCCCTGGCATCAGATGGGTGGT 1093
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
836 AAGCGGCATCTGGGATCATTCTCAAGCACCCCTGGGCTCAGATGGATCGT 885
1094 GAGTTTTTTAGCTTCTTATCTGCCCCCTCATCTGTGTGTAATGTT..... 1137
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
886 GAGTTATCAATCTCCGAAT.....ACATGCTTGTTTTTTATTCTTGCA 928
1138 ..TGGCGTA.....TGGAGTCAGGTGATTT.....ACCTT 1165
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
929 ACTGGCCTAGCTGTTCCAATTCAATCCATATTTTTTGAAAAAAAAAATAT 978
1166 GCCTGTGATGTTTGTTCCTTGTTCAGGTGGCCTTCTTCAGGCAGTTCTTC 1215
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
979 TCATGCCGTGTTTG.....TTGTTAGGTAGCATTCTTCAGGCAGTTCTTT 1023
1216 AGGTCAGTCACCAAGGTGGACTACCTGACCTTGAGGGCAGGCTTCATCAA 1265
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1024 GGGTCCGTCACCAAGGTGGACTACCTGACCATGCGGCAAGGCTTCATCAA 1073
1266 CGTACGTGC....CTCCCTTCTAGCTCCGCCATTGCTGCCGCGATGTAG 1311
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
1074 TGTATATACTAATCAAACCTGACCAATTCAACATTGATGATGC.AAACAG 1122
1312 CAGCAAAGCTTCT.....CAAGTTATCCTTCTGACGCTAAAGTTCCCA 1354
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
1123 AAGACCAGGTTTTTTTTTTTCCGAGTTGTGCAT.TGAAGTTAATG..... 1165
1355 TGTTTTTTCCTCAAATTATTCTGCGCAGGCG.CATTTGTTCGCAAAACAGC 1403
      ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
1166 .GTTTGTAGCTTC...TTCTCTTTTGCAGGCGCCATTGTGTCGAGAATAGC 1211
1404 AAGTTCGACTTCCACAAGTACATCAAGAGGTCGATGGAGGACGACTTCAA 1453
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1212 AAGTTCGACTTCCACAATAACATCAAGAGGTCTTTGGAGGACGACTTCAA 1261
1454 GGTGTCGTCGGCATCAGGTACGTTCCATTCTCTCTGTCACCAGACCA 1503
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1262 AGTTGTCGTTGGCATCAGGTCCG.....TCCTCGCTTT..... 1294
1504 CACCCCATGGATAGATTTTAAACAATTGCTGTCAGGTCCACATGATAACA 1553
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1295 .....ATTAATTATAGGA....CTCTTATATTCAACATTTTTTTT 1330
1554 ATATACTATGA.ACTTGGTCTTTGCTCCTTGTCTTG.....CACGATCA 1597
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1331 ATAAAGAAACATATTTAGTCT...CCAGTTGTGTATGTGTATGTGGATCT 1377
1598 TGACACATTTGGCCTGTTTTCGAGCCTCCCGCTGTGGGGTGTGGCGATC 1647
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1378 TGACACATTTGG.CTGGTTTTCGAGCCTCCCTCTGTGGTTCGTGCGAATC 1426
1648 CTCACCTCTTCTTTCGATCAATGGTATGGACCTTCTCCTCTCCGGTTT 1697
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1427 CTTGTACTCTTCTCGATATCCACGGTA..ATCCTTGTCT....ATT 1469
1698 CTCTATTGCTTTGCAGCTAAATAAAACACTTGCAATTCTGCTCTCGTGATCA 1747
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1470 CATCTTTTTTTTACTCTCAAAACCTTGTTCTGAATTGGTCTTATAATCA 1519
1748 CCGCTCATTTTTCAACCATTTCTTTTTCTACTCATAGGGGTTGGCACGCT 1797
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1520 CCATCGATTTTTTTTCAACTT.TTCCCGCGTGATAGGTCTTGGCACACT 1568
1798 CATCTGGATTCTTTTCATCCCTCTCGTGGTAAGTGC.AGATTTCTCC.AT 1845
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1569 TATTTGGATCTCTTTTGTTCCTCTCATCGTAAGAGCGAAATTTCCCTGT 1618

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FIGURE 6 cont'd

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FIGURE 6 cont'd

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CGCTGCTCCGCCAGCAACACAGACACAGCAGCTACCTGCGT

ACG TAGCGTGCGCTTTCTTTTTTTTCCITTCGCTCTCTGCTGCTCGGCCGGCCACG

TGCATAGCCGGCCACGGCCAGGCACCTCGCGGTTGCGCTGCGGTGCATCTGCGTGTGCGTA

CCTGGTAGAGGCGCCCGTCTGCTTGCTCCGGGCAAGGAAGGAGGTTGCGGCCGTCGACCG

M S D X K K G V P A R E L P E T P S **V** 20  
ATGTCGGACAAAAAGGGGTGCCCGCGCGGAGCTGCGGAGACGCCGCTGCTGGCGCGTG 60

**A V V V F A A M V L V S V L M** E H G L H K 40  
CGCGTGGTCTTCGCGCCCATGGTGCTGCTGCTCGCTCATGGAACACGGCCTCCACAAAG 120

L G H W F Q H R H K K A L W E A L E K M 60  
CTCGGCCATTGGTCCAGCAGCCGCAAGAAAGCCCTGTGGGAGCGCTGGAGAAATG 180

K A E **L M L V G F I S L L I V V T M Q U D A E** 80  
AAGCGGAGCTCATGCTGGTGGCGCTTCATATCCCTGCTCCTCATGCTACGCAGGACGCC 240

**I W I A A K I C I S** E D A A D V M W P C K R 100  
ATCATGCGCAAGATATGCATCTCGAGGATGCGCGGACGTCATGTGGCCCTGCAAGCGC 300

G T E G R K P S K Y V D Y C P E G K V A 120  
GGCACCAGGGCGGCAAGCCAGCAAGTACGTTGACTACTGCGCGAGGGCAAGGTGGCG 360

L M S T G S L H Q L H **V F I F V L L A V V F** 140  
CTCATGTCCAGGGCAGCTTGACACAGCTGCAGCTCTTCATCTTCGTGCTCGCGGCTTTC 420

**H V T T A C C T A C G G C C A T C A C C A T G C T A A G C C G T C A A A A T G A G A A T G G A A G** 160  
CTGTACCTACAGGCTCATCACCATTGCTAAGCCGCTCAAAATGAGAAATGGAAG 480

K W E T E T T S L E T Q F A N D P A R F 180  
AAATGGGAGACAGAGACCACCTCCTTGGAAATACCACTTGCAAATGATCTGCACGGTTC 540

R F T H Q T S F V K R E L G L S S T P G 200  
CGGTTACGCACAGAGCGTGGTTCGTGAAGCGCCACCTGGGCGCTCCAGCACCCCTGGC 600

I R W V V A F F R Q F F R S V T K V D Y 220  
ATCAGATGGGTGGTGGCCTTCTTCAGGCAGTCTTTCAGGTCAGTCACCAAGGTGGACTAC 660

L T L R A G F I N A H L S Q N S X F D F 240  
CTGACCTTGAGGCGAGGCTTCATCAAGCGCATTTGTCGCAAAACAGCAAGTTCGACTTC 720

H K Y I K R S M E D D F K **V V V G G I S L** 260  
CACAAGTACATCAAGAGGTCGATGGAGGACGACTTCAGGTGCTGCTGGCATCAGCCTC 780

**P L W G V A I L L L F L** D I N G V G **T M L** 280  
CGGCTGCGGGTGTGGCGATCCTCACCTCTTCCTTGACATCAATGGGCTTGGCAGGTC 840

**I W I S F I P L V I L L C V G** T K L E M 300  
ATCTGGATTCTTTCATCCCTCTCGTGATCTCTGTGTGTGGAACCAAGCTGGAGATG 900

I I M E M A L E I Q D R A S V I K G A P 320  
ATCATCATGGAGATGGCCCTGGAGATCCAGGACCGGGCGAGCGTCATCAAGGGGGCCGCC 960

V V E P S N K F F W F H R P D W V L F F 340  
GTGGTCGAGCCGCAAGATTCTTCTGTTCCACCGCCCGCTGGGCTGCTCTCTTC 1020

I H L T L F O N A F O M A H F V W T V A 360  
ATACACCTGACGTTGTTCCAGAACCGGTTTCAGATGGCGCATTTTGTGTTGACAGTGGCC 1080

T P G L K K C Y H T Q I G L S I M K **V V** 380  
ACGCCGGCTTGAAGAAATGCTACCACACGCAGATCGGGCTGAGCATCATGAAGGTGGTG 1140

**V G L A D Q F L C S Y M T F P L Y A L E V** 400  
GTGGGGTAGCTCTCCAGTCTCTGCGAGCTATATGACCTTCCCCTCTACGCGCTCGTC 1200

**T** Q M G S N M K R S I F D E Q T S K A L 420  
ACACAGATGGGATCAACATGAAGAGGTCATCTTCGACGAGCAGCTCCAAGGCGCTC 1260

T N W R N T A K E **K K K V R** D T D M L M 440  
ACCAACTGGCGGAACAGGCCAAGGAGAAGAGAAGTCCGAGACACGGACATCTGTATG 1320

A Q M I G D A T P S R G S S P M P S R G 460  
GCTCAGATGATCGGCGAGCACAACCGAGCGAGGCTGTCGCGCATGCGGACCGGGGC 1380

S S P V H L L K K G M G R S D D P Q S A 480  
TCATCACCCGTGCACCTGCTTCACAGGGCATGGGCGGTGCGACGACCCCAGAGCGCG 1440

P T S P R T Q Q E A R D M Y P V V V A H 500  
CCCACCTCGCAAGGACCCAGCAGGAGGCTAGGGACATGTACCCGGTTGTTGGTGGCCAC 1500

P V H R L N P N D R R R S A S S S A L E 520  
CCGTGACAGACTAAATCCTAACACAGGAGGAGTCCGCGCTGCTGCTGGGCCCTCGAA 1560

A D I P S A D F S F S Q G  
GCGGACATCCCAGTGCAGATTTTCCITTCAGCCAGGGATGAGACAAGTTTCTGTATTCA  
TGTAGTCCCAATGTATAGCCAACATAGGATGTATGATTCGTACATAAGAAATACAA  
TTTTACTGAGTC

## Figure 8

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1   GAATTCAATT AAGGACAACA ACGGATGATA GCCTTAAGCT AGAGAGGATT
51  CATATCGATT AATTAAGTGT ACTTAAGTTG AGGTAAACT CTATCGATTG
101 CTTTGGACAC CGGCTCTCCC ATGATCTGCC AAGTTGAGCC GGCCTACCTA
151 ATTTCTTTCG AAAGCACACA ACAAACGAAG GTAACCACTA ATCTAGACAC
201 CACGCCTAAG TTATCAATTA CTACTCTAGT CTCGCGTAGA AACTTCATTG
251 TTTATGGAGA GTGCTAGTAC TAGAGTACTT AATATAATAG TAAGCGACAA
301 ACCCACGACG ATGAGAATGT ACCTCACTTA CGTAGTCAAT TAAGTCGAAA
351 AGGAAATCTT GAACACTTAC TTTATTAAAG AAGTATTCCC CGAGGTACAG
401 GAGAGGAGAG CACGCCAATA ACTCCAGCAC TCCTCCGAAA CCTTCTCAC
451 TCTCTACCCT TTTTCTCCAC ACAACTAAAA TGATGTCTAA TGTATGAAAG
501 TGAGTTGTAC TCTATTTTGT TGTGTGTTTG GAAGTGAAAT TAGCTCATCC
551 TTTTATAGCA ACTTAATGCT CGGTTGTAGG TTGGTAATTA AGTCGGTAAA
601 CACTCACAAC CACCATCGTC AACCATAGG AGATCGCCAC ATGATCGAAA
651 GCTGACAGTT AGGGGTGCCA ACCCTGTTTT GTCCGAACCA AGCAAACAAC
701 CTCTATCTAG GACCTCTCTT CTATCTCTGA CAAGTCGGCC CATATGGCGG
751 TGCACATATG ATTAAGTCAA TTTCAGTCGT TTTGGACTGT CATGTGGGCC
801 CTTCCAATCC TTGTGCTCCC ATATGATTGG TCGAAAGTAC ATTTAATTCC
851 TGGGTGAGTG CTAGAACTAA TATGATAGAT GTGCTCCGGC TCCTGGGAAA
901 GAGGCCACTT GACATACTTG GGGTAGTGCC CCAAGGGTAT TCCCTATCGC
951 TTTTTCATAA TTTTCTCTCT CCAAAATCGG ACGGAAACAA TAAAAAGAG
1001 AGGUGATGTT CATCGGCAA TATCTATTTT TTTGATAGTG TCTTCCCTTA
1051 AAACTTGATT TTTGCGAAGA CTTCGGGCTA AAACCATGPA ATCAGAGTTC
1101 CTTGTAACAA ATTTAATTTG CCTAAATACA AAAAAGATCG AATGGAGATA
1151 GCATTAAACT TGCTCCATAC GAATCATATT AGTTGGACCG TAACTCATAG
1201 AAAAAGTTGC AAGTTGGTTG ACCTATCAAC CCTCTTATGT TGACCGTAAA
1251 CCTGTTATGC ATTAAGGATT AAGTACCGGC AGATCGTCAC TACTCACGAA
1301 TGCACAAATT TCCGGTAACG TAGGATGGGA TGAGTTGGTC AGAAACGGGT
1351 CACCACGTCG CCCAACCTGC CGCGATCGAG CCATTGGCCG GCGATGCACG
1401 CGCTTTGACA CAGCCGCCCG CGCCCCCGG GCCCCCCCC GTTTTTAATA
1451 AAAACCGGCC GCCCCCTGTC AAAGGTGTCA AAGTGTCAAG TGCATCAGAG
1501 CTAAGCTAGC GGTCAACCAG TCAGCTCACC CCGAGACGCA CCAGGGGATC
1551 TATUGGATCA TGGCAGGTGG GAGATCGGGA TCGCGGGAGT TGCCGGAGAC
1601 GCCGACGTGG GCGGTGGCCG TCGTCTGCGC CGTCCTCGTG CTCCTCTCCG
1651 CCGCATGGA GCACGGCCTC CACAACCTCA GCCATGTACG CGCGCGCGCA
1701 CGCGGTGTGC TCATCTCTCG AGTTAATTTG GTTGTGTTG TTGTTGTGTT
1751 CTTGTGACAT CTCATTAAAC ATCCGATCGT GGTGATCGA TCGCCCTGTG
1801 GTEGCGATAC TGCTTGCAAT GCAGTGCTTC CGTAGGCGGC AGAAGAAGGC
1851 CATGCGCGAC GCCCTCGACA AGATCAAAGC AGGTCACCCT CAGCCTCAGC

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**FIGURE 8 cont'd**

1901 TCACCCCTCAG CCTCCATCTC TAAATATTTG ACGCCGTTGA CTTTTTTAAA  
1951 TATGTTTGAC CATTCGTCTT ATTTAAAAAA TTTAAGTAAT TATTAATTCT  
2001 TTTTCTACCA TTTGATTTCAT TGCTAAATAT ACTATTATGT ATACATATAG  
2051 TTTTACATAT TTCACTAAAG TTTTTTAAATA AGACGAATGG TCAAACATGT  
2101 TTAAAAAAGT CAACGGCGTC AAACATTTAG GAAGAAGAGA ATATTATATT  
2151 GCTGCTCCCC TCTAGCCACT TTGCTGCCTC CCTCGTCATT TTTTCAAGTA  
2201 TTTTACGCAA GACTGGTCCT CCAAATCAAA CGTCACAAAT AAGCCATTTA  
2251 TAGTTTCCTT TCGCTTTTTA AGGGGGACTA CTTGTATTTA ATCATGGAGG  
2301 AAACACCAG TCGGATGTCC GATTACTTAA AAAAAAATC GGGGGACTAA  
2351 TTTTTTTGGC TGATCATCGG TGAAATATTA GGTATATATAT GTTGAAAAAA  
2401 AATCAGCCAC AAACAATGAA ATATTTTGTG AAACACATAT TAGACACGTT  
2451 GAAACGTATC ATTGTTACGT ATAAAACATC GAATGTTAAC AGATTAAAAC  
2501 ATATGTTTTT TTTTAATCAG AATATAATCA TGCGATATAT TATTGTAAAG  
2551 ATATAATTAC AACGAATACA ACAGTGCAT CGGATTATAT ATATATTAGT  
2601 AGTTTAAGAG AAAAATCATT TTGAAGATTA CTAGATACAT ACACGTATAG  
2651 ATGGATGAAG TGGAGAGAGA TTAGAGATAA GTAGTTATAT GAATTTTGTG  
2701 AAACACACTT AAGACATATG TTCAAACATA CTGCTATTAT GTATGAAATA  
2751 TTGAGTTTTA ACGGTTTAAA ACACATATTC TTTTAATTAG AATGTAATAA  
2801 TGTGATATCT TGTTGTAAAA TTTAATTACA TCTAATATAA CGGTGTGATT  
2851 AGATTGTATG TTGGATAACA TGCCCATCGG TTGGCTTATT TAGGGAATAA  
2901 GCCAAATGGT ATATTTGCAA ACGAAAAATA ATTTGTAAAT AAAACTTTTA  
2951 TGTATGTATT CTTAACGATC TAGCAGCAA GGCTGAAAAA TAAACTTCGA  
3001 TGAAAAATCT CAAAATCAAC TCTTAAATTT TAAATTTTGG CTTATAAGTA  
3051 TAGTTCCTAA CTAGTTTAGA AGAAAAATA TTTAAAGCGG GGAAGAGGAA  
3101 AAGGAATAAA CTAATAGCTA AATTATTGCA TGCATGTAGC GATTTGAGGA  
3151 CGACCGAGTT GTTTTGTCTG GATCAGCCGA CCGAGACAGA GCAATCTTCT  
3201 TTAATCATAA ATAACCAGAA AAACCATACC AGTTCATCAC AATGGACCGA  
3251 GTCAGAGTCA TTACATATTT TTCATTGTTG CGCACAGGAT TCACCATGTT  
3301 CTTATGGGAA ATATTTTAA CTCTCAAATG GTTATGATTT TGAACCTCTCA  
3351 TTTTGTAGAG AGAATTAACA AGCGAGCGAG CAATCAGGCC AAAAAGGGAG  
3401 AAAGAAAATT ATTTTGTGTA ATTTTPTTTT AAGGTAGGGT GGAGGAGTCA  
3451 TTACATGATT TTTTTTTATA TTCCCTCGTT GATTATATGC TGTTCAAATG  
3501 GTTATGATTT TTTTAAAGA TAACAACAAT ACAAATTAGT ATGTGATAGA  
3551 TCATTTACAG AGCATATAGG ATTAATTTA ACTTCTGTAA ATTACAAAAC  
3601 AAACAAGTTT AACTGTTAAT ATACATTAAA TTTGTTTTTT TCAACTTAGG  
3651 AATTGAATTT TATGTATATA TTTGTAAAT GATATATTAA TTTATTTTTT  
3701 TAAAAAATA ATTATTTAGA TAACACGCAA ACTAGAAAAAC CACCGCAGAA  
3751 GTTCTCATAT TTCTTGTCCT ATCTGCACTT GCAGAGCTGA TGCTGCTGGG  
3801 CTTCATATCC CTGCTTCTCA CCGTGGCACA GCGCCCATC TCCAAGATCT

**FIGURE 8 cont'd**

3851 GCATCCCCAA GTCGGCTGCC AACATCTTGT TGCCGTGCAA GGCAGGCCAA  
3901 GATGCCATCG AAGAAAGAAG CAGCAAGTGG TCGCCGGTCC TTGGCCGGCG  
3951 CCGGCGGCGG GGACTACTGC TCGAAATTCG ATGTGAGAAT AACACCAGCT  
4001 GCCGGAAGC ACAACCTCGA TGCAATAACT AATTTAACTA TAATTGATTT  
4051 TTCTTGGGTT TTCTGCAGGG CAAGGTGGCG CTGATGTCGG CAAAGAGCAT  
4101 GCACCAGCTG CACATTTTCA TCTTCGTGCT CGCCGTGTTC CATGTTACCT  
4151 ACTGCATCAT CACCATGGGT TTAGGGCGCC TCAAAGTGAG TTTGTCGTTC  
4201 TGTCCCTCAT GCACATGTTT TCTCTAGTTC TAGCAAGATT GTCAGTCCTT  
4251 CAAATGGATT GTTTCGACAA GAAACCCAAT TTATTAATTT GCCAGTAAAT  
4301 ATATAATAAT TGATCTTTCT TGGTTTTAGA TGAAGAAATG GAAGAAGTGG  
4351 GAGTCACAGA CCAACTCATT GGAGTATCAG TTCGCAATCG GTAGTGAATT  
4401 AAGAATCTCC CTAACATTTT CATTTAGAA CCTTTATGAT AATGCTTTGA  
4451 AAGAGGAGGA GCAAATCAGC TGAAAAATAT GATCGATCCA TGCAGATCCT  
4501 TCACGATTCA GGTTCACGCA TCAGACGTCG TTCGTGAAGC GGCATCTGGG  
4551 ATCATCTCA AGCACCCCTG GGCTCAGATG GATCGTGAGT TATCAATCTC  
4601 CGAATACATG CTTGTTTTTT ATTCTTGCAA CTGGCCTAGC TGTTCGAATT  
4651 CAATCCATAT TTTTGTAAAA AAAAAATATT CATGCCGTGT TTGTTGTTAG  
4701 GTAGCATTCT TCAGGCAGTT CTTTGGGTCC GTCACCAAGG TGGACTACCT  
4751 GACCATGCGG CAAGGCTTCA TCAATGTATA TACTAATCAA ACCTGACCAA  
4801 TTCAACATTG ATGATGCAA CAGAGACCAG GTTTTTTTTT TCGAGTGTGC  
4851 ATTGAGTAAT GGTTTTAGCT TCTTCTCTTT TGCAGGCGCA TTTGTGCGAG  
4901 AATAGCAAAGT TCGACTTCCA CAAATACATC AAGAGGTCTT TGGAGGACGA  
4951 CTTCAAAGTT GTCGTTGGCA TCAGGTCCGT CCTCGCTTTA TTAATTATAG  
5001 GACTCTTATA TTCAACATTT TTTTATAAAA GAAACATATT TAGTCTCCAG  
5051 TTGTGTATGT GTATGTGGAT CTTGACACAT TTGGCTGGTT TTGCAGCCTC  
5101 CCTCTGTGGT TCGTCGGAAT CCTGTGACTC TTCCTCGATA TCCACGGTAA  
5151 TCCTTGTCTT ATTTCAATCT TTTTCTTACT CTCAAACCT TGTCTGAAT  
5201 TGGTCTTATA ATCACCATCG ATTTTTTTTC AACTTTTTTC CCGCGTGTAG  
5251 GTCTTGGCAC ACTPATTTGG ATCTCTTTTG TTCCTCTCAT CGTAAGAGCG  
5301 AAATTTCCCT GTCCAAAGAA ACAGTTAACA TAATTAATTA TGCTTTAATT  
5351 TATCATGAAA ATTAATATGA TCATATAACT AATGAACAAA CATTCATGTG  
5401 AATGCCACCG TTGTCTCAGA TCGTCTTGTT AGTTGGGACC AAGCTAGAGA  
5451 TGGTGATCAT GGAGATGGCC CAAGAGATAC AGGACAGGGC CACTGTGATC  
5501 CAGGGAGCAC CTATGGTTGA ACCAAGCAAC AAGTACTTCT GGTTCACCG  
5551 CCCTGACTGG GTCTTGTCTT TCATACACCT GACACTCTTC CATGTACATG  
5601 TTTAAACCT AAACCTTGCT GCTCAACTAC AAATAGTACT TTATCTTTCA  
5651 CAATTAACAC CTAATTAAC AACATAGCAT CCATCCATTT GTGGCTACTG  
5701 ATCGATGGGA CGACGGATCG ATCATCACC GAACGCATTT CAGATGGCGC  
5751 ATTCGTATG GACTATGGTG TGTATGCTAC TTGCTTAGTT GTTGCCATTA

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**Figure 8 cont'd**

5801 TCAGTTCTTA AGCAAATTAA GTGTGATGCA TGCCTGACT AATGAGACAA  
5851 AAAATGACAC AGCTTGTTCA TCGATCTGGT TGTMTTGTGT GTGACAGGCA  
5901 ACACCTGGTC TGAAGAAATG CTTCCATGAA AATATTTGGC TGAGCATCGT  
5951 GGAAGTCATT GTGGGGATCT CTCTTCAGGT GCTATGCAGC TACATCACCT  
6001 TCCCGCTCTA CGCGCTCGTC ACACAGGTGA ACAAGCCATT CACAAATTCT  
6051 ATTAGCCGTT TCTTAATTGA TGACACTGTT AATTTTTAGA CACACGTTTT  
6101 GACCATTTGT CTTATTAAAA ATATTTATGT AATTATCATT TGAGTTGTTT  
6151 TATCACTAAA AGTACTTTTT AAATAATTTA TATTTTGCAT TTGTACAATT  
6201 CTTTTAATAA GATAATGGTC AAACATGTGT CCAAAAGTTA ACAGCATCAT  
6251 CTATTAAGAA AAGGAGGGGT TTTTTTTTTT TGAATTTTG CAAAATTGT  
6301 TCAAAATCAG TCCAAAACCT TTTTTTTTTT CGAATTTCA GTTTCACCTAC  
6351 CAGTCCCAT AAAATGTCTT TTCTTTATTT CCACAAGATT GAACCCATGA  
6401 GATGCCCTTT GTGTTGGTAT GTGTTTTGGC CATCACTTGC AGATGGGATC  
6451 GAACATGAAG AAGACAATTT TCGAGGAGCA AACGATGAAG GCGCTGATGA  
6501 ACTGGAGGAA GAAGGCGATG GAGAAGAAGA AGGTCCGGGA CGCCGACGCG  
6551 TTCTTGGCGC AGATGAGCGT CGACTTCGCG ACGCCGGCGT CGAGCCGGTC  
6601 CGCGTCGCGG GTGCACCTGC TGCAGGTCAC AGGGCGGGTC GGACGCCCCG  
6651 CGAGCCCAAT CACGETGGCC TCACCACCGG CACCGAGGGG GACATGTACC  
6701 CGGTGCCGGC GCGGGCTCGG TCTCGCCAGC TGCTAGACGA CCCGCCGGAC  
6751 AGGAGGTGGA TGGCATCCTC GTCGCGCGAC ATCGCCGATT CTGATTTTTC  
6801 CTTCAGCGCA CAACGGTGAC GGGGGCGATC GGTTCCTGTA TTGATGCTGT  
6851 ACCAAACATA GGAGTTTAAT ATATATATAA TTGTTACGGT AAAATCTAAT  
6901 TATGTGCGC GCACTTATAT TAGTCTTATA GCGCGACTGG TTCGTGATTA  
6951 GACAAGGTGA TGCATGCTGT TTAGTTATAA AGGATATCAG CGCAGCTAAA  
7001 AAAACTTACT CCCTACTTAA TAGATGACCT CGTTGATTTT TAACATTATT  
7051 CGTCTTATTT AAAAATTATA TGCAAATGTT TAAAACATAA ATCATGCTTA  
7101 AAGTACTTTT AGTGATAAAA CAACCTTACAA CAAAATAAAT TATACTTACC  
7151 TAATTTTTTT TAATAAATCG AATCG

1 TTATACCATG TGAGAAAGGC TGAAGCATA TGCTCTTAGC AGGGACGCGT  
51 GCATGTTTAT ATAGGAGGCA TAAGCCGAAG AGATATACAT GAGGAGAGGT  
101 TTAAGATCAG TCTATCTTAT TTACAGTTTA AACACAAGGA GATAGAAAGA  
151 GATCCTAACC TACACATGTT ATACAAGTCA CGTATAATAC AAGAGTTATT  
201 TCGTCTAACA CCCTCCCCCTC TGATATGATA AGTCGCCGGG AGAGAGAGAG  
251 AGTGTGTGGC TGCCCTCGCT GCACTGCACG CACATGTTTA CTTCTCCGAC  
301 TGAAACCACG GTGAAACCGG CGGCGGTGTC GCACTCCCCCT GACTTTCCTC  
351 GCCGGGGTCC CGTCCGGACA ATTAAACCGT CTGTACCTGC CGGGCGTCTGA  
401 CCCGATCGTG ATGTGGCGCC GCTTTGTCTG CAGCGAGCTG CGTGGCCGAT  
451 GGCAACAAAA CTGCGGTCAC ATACATGCAT ACCCCGCATA CCCCAGCGCT  
501 CACCAGTAAG TAGGCTGTGG TGCGGCACCA CGGGCTCGCC GCCATTTCATG  
551 CCATGCATGG GCCACCCGCC GCGGAAACCG CGGCGCTGCT GCCTGCCACC  
601 CCGCCGCCGT TGACGAAGAC TTCGCCCGGC CATCCATAAA AGCATGCATG  
651 GCTTGCTCTC ACCGGTCCGG CCACACACAC CACACTTCAC TTCGCCATTC  
701 GCACCACCGA GAGCGTAGCG TAACGTGTGT TTGAAGTCCT ACCATTAATT  
751 TTGCTGGATC GATGGCTGGG CCGGCGGGAG GTCGGGAGCT GTCGGACACG  
801 CCGACGTGGG CGGTGGCGGT AGTCTGCGCC GTCATGATAC TCGTCTCCGT  
851 CGCCATGGAG CACGCGCTCC ACAAGCTCGG CCACGTACGT GCTCTCGGTT  
901 CACTAGTGCT TAACTGTTTTT TGATGTTTTT GGGCGTGTTT GGTAGCCTGC  
951 ATGGAGAGTG TATGAGCCCA AAAGTTCCTT CCCCAGCCCA CTTTTCGCTG  
1001 TTTGGTAGGG TGTATGGGCT GAGGAGAGCA TGCATCAACT GATGCAAAAA  
1051 GGGCCTCAGC ATAGCTGAGC CCAGCACCCC CGCAGAGGCG AGCTGAGGCG  
1101 AGTTATGCTG AGCCCATGCA CCCTCGCCCC GTCGCCCCGT CGCCCCGTGC  
1151 CTCCCCCCCCT GCACCTCTTC CTCTCCCTC TTCTTACCAA ACACAGTCTC  
1201 ATCCAAACAT GTAACAACAC ATGCATGACC ACCAAACAAC TGAAGATGAA  
1251 TGTATTTCATC ATGTCTATAC TTACCATGCA TCAACAGGGA ACAACTATGC  
1301 TAGGGTGAGA ACAGCTGCCA AACACACCCG TGCACCTACT CATGCTGTGC  
1351 CGGCGCTGGC GTACGTGTGC AGTGGTTCCA CAAGTGGCGC AAGAAGGCCC  
1401 TGGGGGAGGC GCTGGAGAAG ATGAAGGCGG AGCTCATGCT GGTGGGCTTC  
1451 ATATCCCTGC TCCTCATCGT CACGCAGGAT CCCGTCTCCA GGATCTGCAT  
1501 CTCCAAGGAG GCCGGCGAGA AGATGCTCCC GTGCAAGCCT TACGACGGCG  
1551 CCGGCGGTGG CAAAGGCAAG GACAATCACC GGAGGCTTCT CTGGCTCCAA  
1601 GGCAGAGCG AGACCCACCG CCGGTTCCCTG GCTGCCCCGG CCGGAGTGGA  
1651 CGTCTGCGCC AAACAGGTGA GCACCTAGCG TCGCCACAAA CCACAAACTA  
1701 GCTAATGAGC ATGGACCTGA ATTTCTTCTC TTCTTGCTT GGCTTGACTA  
1751 AATTGGTTGT GCAGGGCAAG GTGGCGCTGA TGTCAGCGGG AAGCATGCAC  
1801 CAACTGCACA TATTCATCTT CGTGCTCGCC GTCTTCCACG TCTTGATACG  
1851 CGTCGTCACC ATGACCCTAA GCCGTCTCAA AGTGAGCATC ATACTCGAGC

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1901 TGT TTGTCAA TAATCCTTGG TTTCCAATCC AATTCCAAAG CTGGCACTGA  
1951 TCCTGCTCCG GCTTCCTGCA GATGAAGCAA TGGAAGAAGT GGGAGTCGGA  
2001 GACCGCCTCG CTGGAGTATC AGTTCGCGAA TGGTCAGCTT CAACTTTTCT  
2051 TACTGAAACC GGATGCATTT ACAACAAACG CACGCACGAT CAATCATCAC  
2101 AGTGTGAGCC GATACGTTGA ACCGATTGAA TCCTCGCAGA TCCATCGCGG  
2151 TGCCGGTTCA CGCACCAGAC GACGTTGGTG AGGCGGCACC TGGGCCTCTC  
2201 CAGCACCCCC GCGTCAGAT GGGTGGTGGC CTTCTTCAGG CAGTTCTTCA  
2251 CGTCGGTGAC CAAGGTGGAC TACCTGACCT TGCGGCAGGG CTTCATCAAC  
2301 GCGCATCTCT CGCAGGGCAA CAGGTTCGAC TTCCACAAGT ACATCAAGAG  
2351 GTCGTTGGAG GACGACTTCA AAGTCGTCGT CCGCATCAGG TACGCGCCAT  
2401 TCCTTTCTCT GCACAAATTA ATACATCCAC CACCACATAG GTAGATAGAT  
2451 AGATCGATAG ATAGATTATA CAAGTGCCGG TACGTACGTA CGTCTCATAT  
2501 GATCTTGACA CATCTGTCTT CTTGCCGCAG TCTCAAGCTC TGGTTCGTGG  
2551 CGGTCCTCAT CCTCTTCCTT GATTTTCGAC GTAGCCGCCT TGTCCATGCC  
2601 CTGCTCGCCC TCTCCTCCGC TTCTCTCCAT AATTTGTGAA CTTGTCCCGT  
2651 ATATAACCAC ACCACCGTCG TCTTCTCGCA GGGATCGGCA CTCTTCTCTG  
2701 GATGTCCGTG GTTCCTCTCG TGGTAAGTCC ACAATTTGAA TAGACAACCT  
2751 GTCCAATTGT GATGTACAGT ACCTCCAAAC TTAATTAACA TGTCATTTGC  
2801 TGATGTCTTG CGTGTAACAT TAGATCCTCT TGTGGGTGG GACCAAGCTG  
2851 GAGATGGTGA TCATGGAGAT GGCCCAGGAG ATCCATGACC GGGAGAGCGT  
2901 CGTCAAGGGT GCTCCCGCCG TCGAGCCCAG CAACAAGTAC TTCTGGTTCA  
2951 ACCGGCCTGA CTGGGTCTCT TTCTCATGC ACCTCACACT CTTCCAGAAC  
3001 GCGTTTCAGA TGGCTCATTT CGTGTGGACA GTGGTACGTA CAAGTACTTG  
3051 TCACTTCACT TAGGCTAACT CCAACAAACG ACCCCAAATT AATGGTCCGT  
3101 CGCGTCTGTT TGGGGTATGT TTGGGGTAAA CGGACACAAA ACTCAATCCA  
3151 ACGCGCGGTA GCAAACGAAC GTTTTTCGGT ACGTTTTCGT CCGCTTTCGC  
3201 CCCATCCCAG CCCAAATTCT TTGACGTTGT TGCATCGCAG GCCACGCCCG  
3251 GCTTGAAGAA ATGCTACCAC GAGAAAATGG CAATGAGCAT CGCCAAGGTC  
3301 GTGCTGGGGG TAGCCGCCCA GATCTGTGTC AGCTACATCA CCTTCCCGCT  
3351 CTACGCGCTC GTCACGCAGA TGGGCTCACA CATGAAGAGA AGCATCTTCG  
3401 ACGAGCAGAC GGCCAAGGCG CTGACCAACT GCGGAAAGAT GGCCAAGGAG  
3451 AAGAAGAAGG CCCGAGACGC GGCCATGCTG ATGGCGCAGA TGGGCGGCGG  
3501 CGCGACGCCG AGCGTCGGCT CGTCGCCGGT GCACCTGCTC CACAAGGCCG  
3551 GGGCGCGGTC CGACGACCCC CAGAGCGTGC CGGCGTCCCC GAGGGCCGAG  
3601 AAGGAAGGCG GCGGCGTGCA GCATCCGGCG CGCAAGGTAC CTCCTTGTGA  
3651 CGGGTGGAGG TCGGCCTCGT CGCCGGCGCT CGACGCTCAC ATCCCCGGTG  
3701 CAGATTTTGG CTTACGACAG CAACGTTGAC CGATCAGACA AGTTCCTTTT  
3751 TTTTTCGGTG AATAGAAGCG TATCATTTCA TTGATAGACA GTAGAAATTA  
3801 CAGGAATGGC TGTCTACTA CTATGTACAC AAGGGCACAG CAAAGGATCA

**Figure 9 cont'd**

3851 TTGATCTTGT TACAAGAGCA GTAGAAAGGG ATTGCTCTCC ATTGATCTTG  
3901 TTAAGTTGTA TGTCACAAT TGTTCAGAA AAAAGTGTAT GTCAATCCCA  
3951 CCAAGAGCTG AGTTTGTGAT GATTTCGTGCA ATAAGAATTG CAAGTTTCAC  
4001 CGACTCAAAA ATGAAGCTTC TAAGTACGCA CCAACCAACG GACTCTTCA  
4051 TCTCAACAAA AGAACTGTAA ATGGCAATAA TTCTGATAAC ATCGGAAGGG  
4101 AGCTC

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**Figure 10**

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1  ATGGCAGGTG GGAGATCGGG ATCGCGGGAG TTGCCGGAGA CGCCGACGTG
51  GGCGGTTGGCC GTCGTCTGCG CCGTCCTCGT GCTCGTCTCC GCCGCCATGG
101 AGCACGGCCT CCACAACCTC AGCCATAAAA CCACCGCAGA AGTTCTCATA
151 TTTCTTGTC TATCTGCACT TGCAGAGCTG ATGCTGCTGC GCTTCATATC
201 CCTGCTTCTC ACCGTGGCAC AGGCGCCCAT CTCCAAGATC TGCATCCCCA
251 AGTCGGCTGC CAACATCTTG TTGCCGTGCA AGGCAGGCCA AGATGCCATC
301 GAAGAAGAAG CAGCAAGTGG TCGCCGGTCC TTGGCCGGCG CCGCGGGCGG
351 GGACTACTGC TCGAAATTCT ATGGCAAGGT GCGCGTGATG TCGGCAAAGA
401 GCATGCACCA GCTGCACATT TTCATCTTCG TGCTCGCCGT GTTCCATGTT
451 ACCTACTGCA TCATCACCAT GGGTTTAGGG CGCCTCAAAA TGAAGAAATG
501 GAAGAAGTGG GAGTCACAGA CCAACTCATT GGAGTATCAC TTCGCAATCG
551 ATCCTTCACG ATTCAGGTTT ACGCATCAGA CGTCGTTCTG GAAGCGGCAT
601 CTGGGATCAT TCTCAAGCAC CCCTGGGCTC AGATGGATCG TAGCATTTCT
651 CAGGCAGTTC TTTGGGTCCG TCACCAAGGT GGACTACCTG ACCATCGGGC
701 AAGGCTTCAT CAATGCGCAT TTGTCGCAGA ATAGCAAAGT CGACTTCCAC
751 AAATACATCA AGAGGTCTTT GGAGGACGAC TTCAAAGTTG TCGTTGGCAT
801 CAGCCTCCCT CTGTGGTTCT TCGGAATCCT TGTACTCTTC CTCGATATCC
851 ACGGTCTTGG CACACTTATT TGGATCTCTT TTGTTCTCT CATCATCGTC
901 TTGTTAGTTG GGACCAAGCT AGAGATGGTG ATCATGGAGA TGGCCCCAGA
951 GATACAGGAC AGGGCCACTG TGATCCAGGG AGCACCTATG GTTGAACCAA
1001 GCAACAAGTA CTTCTGGTTC AACCGCCCTG ACTGGGTCTT GTTTTTCATA
1051 CACCTGACAC TCTTCCATAA CGCATTTCTG ATGGCGCATT TCGTATGGAC
1101 TATGGCAACA CCTGGTCTGA AGAAATGCTT CCATGAAAT ATTTGGCTGA
1151 GCATCGTGGA AGTCATTGTG GCGATCTCTC TTCAGGTGCT ATGCAGCTAC
1201 ATCACCTTCC CGCTCTACGC GCTCGTCACA CAGATGGGAT CGAACATGAA
1251 GAAGACAATT TTGAGGAGC AAACGATGAA GGCGCTGATG AACTGGAGGA
1301 AGAAGGCGAT GGAGAAGAAG AAGGTCCGGG ACGCCGACCC GTTCTCTGGC
1351 CAGATGAGCG TCGACTTCGC GACGCGGGCG TCGAGCCCGT CCGCCTCGCC
1401 GGTGCACCTG CTGCAGGTCA CAGGGCGGGT CGGACGCCCG CCCGAGCCAA
1451 TCACGGTGGC CTCACCACCG GCACCGGAGG AGGACATGTA CCCGGTGCCG
1501 GCGGCGGCTG CGTCTCGCCA GCTCCTAGAC GACCCGCCGG ACAGGAGGTG
1551 GATGGCATCC TCGTCGGCCG ACATCGCCGA TTCTGATTTT TCCTTCAGCG
1601 CACAACGGTG A

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## Figure 11

```

1  ATGGCTGGGC CGGCGGGAGG TCGGGAGCTG TCGGACACGC CGACGTGGGC
51  GGTGGCGGTA GTCTGCGCCG TCATGATACT CGTCTCCGTC GCCATGGAGC
101 ACCCGCTCCA CAAGCTCGGC CACTGGTTCC ACAAGTGGCG CAAGAAGGCC
151 CTGGGGGAGG CGCTGGAGAA GATGAAGGCG GAGCTCATGC TGGTGGGCTT
201 CATATCCCTG CTCCTCATCG TCACGCAGGA TCCCGTCTCC AGGATCTGCA
251 TCTCCAAGGA GGCCGGCGAG AAGATGCTCC CGTGCAAGCC TTACGACGGC
301 GCCGGCGGTG GCAAAGGCAA GGACAATCAC CGGAGGCTTC TCTGGCTCCA
351 AGCGGAGAGC GAGACCCACC GCCCGTTCTT GGCTGCCCCG GCCGGAGTGG
401 ACGTCTGCGC CAAACAGGGC AAGGTGGCGC TGATGTCAGC GGAAGCATG
451 CACCAACTGC ACATATTTCAT CTTCTGCTCT GCCGTCTTCC ACGTCTTGTA
501 CAGCGTCTGC ACCATGACCC TAAGCCGTCT CAAAATGAAG CAATGGAAGA
551 AGTGGGAGTC GGAGACCGCC TCGCTGGAGT ATCAGTTCCG GAATGATCCA
601 TCGCGGTGCC GGTTCACGCA CCAGACGACC TTGGTGAGGC GGCACCTGGG
651 CCTCTCCAGC ACCCCCGGCG TCAGATGGGT GGTGGCCTTC TTCAGGCAGT
701 TCTTCACGTC GGTGACCAAG GTGGACTACC TGACCTTGCG GCAGGGCTTC
751 ATCAACGCGC ATCTCTCGCA GGGCAACAGG TTCGACTTCC ACAAGTACAT
801 CAAGAGGTCG TTGGAGGACG ACTTCAAAGT CGTCGTCCGC ATCAGTCTCA
851 AGCTCTGGTT CGTGGCGGTC CTCATCTCTT TCCTTGATTT CGACGGGATC
901 GGCACCTCTT TCTGGATGTC CGTGGTTCTT CTCGTGATCC TCTTGTTGGT
951 TGGGACCAAG CTGGAGATGG TGATCATGGA GATGGCCCAG GAGATCCATG
1001 ACUGGAGAG CGTCGTCAAG GGTGCTCCCG CCGTCGAGCC CAGCAACAAG
1051 TACTTCTGGT TCAACCGGCC TGA CTGGGTC CTCTTCTTCA TGCACCTCAC
1101 ACTCTTCCAG AACGCGTTTC AGATGGCTCA TTTCGTGTGG ACAGTGGCCA
1151 CGCCCGGCTT GAAGAAATGC TACCACGAGA AAATGGCAAT GAGCATCGCC
1201 AAGGTCGTGC TGGGGGTAGC CGCCAGATC TTGTGCAGCT ACATCACCTT
1251 CCCGCTCTAC GCGCTCGTCA CGCAGATGGG CTCACACATC AAGAGAAGCA
1301 TCTTCGACGA GCAGACGGCC AAGGCGCTGA CCAACTGGCG AAAGATGGCC
1351 AAGGAGAGAA AGAAGGCCCC AGACGCGGCC ATGCTGATGG CGCAGATGGG
1401 CGGCGGCGCG ACGCCGAGCG TCGGCTCGTC GCCGGTGCAC CTGCTCCACA
1451 AGGCGGGGGC GCGCTCCGAC GACCCCAAGA GCGTCCCGGC GTCCCCGAGG
1501 GCGGAGAAGC AAGGCGGCGG CGTGCAGCAT CCGGCGCGCA AGGTACCTCC
1551 TTGTGACGGC TGGAGGTCCG CCTCGTCCGC GCGCTCGAC GCTCACATCC
1601 CCGCTGCAGA TTTTCGCTTC AGCACGCAAC GTTGA

```

1 GTTGGTACAT AAAAGACTCT TCCTTTGTCT GTTTTTTGTT CCCAGATTCA  
51 TCTTTACTTA TTGACTAAAT TCTCTCTGGT GTGAGAAGTA AAATGGGTCA  
101 CGGAGGAGAA GGGATGTCGC TTGAATTCAC TCCGACGTGG GTCGTCGCCG  
151 GAGTTTGTAC GGTCATCGTC GCGATTTTAC TGGCGGTGGA GCGTTTGCTT  
201 CACTATTTTCG GTACTGTTCT TAAGAAGAAG AAGCAAAAAC CCCTTTACGA  
251 AGCCCTTCAA AAGGTAAAG AAGAGCTGAT GTTGTTAGGG TTTATATCGC  
301 TGTTACTGAC GGTATTCCAA GGGCTCATTT CCAAATTCCTG TGTGAAAGAA  
351 AATGTGCTTA TGCATATGCT TCCATGTTCT CTCGATTCAA GACGAGAAGC  
401 TGGGGCAAGT GAACATAAAA ACGTTACAGC AAAAGAACAT TTTCAGACTT  
451 TTTTACCTAT TGTTGGAACC ACTAGGCGTC TACTTGCTGA ACATGCTGCT  
501 GTGCAAGTTG GTTACTGTAG CGAAAAGGGT AAAGTACCAT TGCTTTCGCT  
551 TGAGGCATTG CACCATCTAC ATATTTTCAT CTTCGTCCCTC GCCATATCCC  
601 ATGTGACATT CTGTGTCCTT ACCGTGATTT TTGGAAGCAC AAGGATTCAC  
651 CAATGGAAGA AATGGGAGGA TTCGATCGCA GATGAGAAGT TTGACCCCGA  
701 AACAGCTCTC AGGAAAAGAA GGGTCACTCA TGTACACAAC CATGCTTTTA  
751 TTAAAGAGCA TTTTCTTGGT ATTGGCAAAG ATTCAGTCAT CCTCGGATGG  
801 ACGCAATCCT TTCTCAAGCA ATTCTATGAT TCTGTGACGA AATCAGATTA  
851 CGTGACTTTA CGTCTTGGTT TCATTATGAC ACATTGTAAG GGAAACCCCA  
901 AGCTTAATTT CCACAAGTAT ATGATGCGCG CTCTAGAGGA TGATTTCAA  
951 CAAGTTGTTG GTATTAGTTG GTATCTTTGG ATCTTTGTCTG TCATCTTTTTT  
1001 GCTGCTAAAT GTTAACGGAT GGCACACATA TTTCTGGATA GCATTTATTC  
1051 CCTTTGCTTT GCTTCTTGCT GTGGGAACAA AGTTGGAGCA TGTGATTGCA  
1101 CAGTTAGCTC ATGAAGTTGC AGAGAAACAT GTAGCCATTG AAGGAGACTT  
1151 AGTGGTGAAA CCCTCAGATG AGCATTTCTG GTTCAGCAAA CCTCAAATTG  
1201 TTCTCTACTT GATCCATTTT ATCCTCTTCC AGAATGCCTT TGAGATTGCG  
1251 TTTTCTTTTT GGATTGGGT TACATACGGC TTCGACTCGT GCATTATGGG  
1301 ACAGGTGAGA TACATTGTTC CAAGATTGGT TATCGGGGTC TTCATTCAAG  
1351 TGCTTTGCAG TTACAGTACA CTGCCTCTTT ACGCCATCGT CTCACAGATG  
1401 GGAAGTAGCT TCAAGAAAGC TATATTCGAG GAGAATGTGC AGGTTGGTCT  
1451 TGTTNGTTGG GCACAGAAAG TGAAACAAAA GAGAGACCTA AAAGCTGCAG  
1501 CTAGTAATGG AGACGAAGGA AGCTCTCAGG CTGGTCCTGG TCCTGATTCT  
1551 GGTCTTGTT CTGCTCCTGC TGCTGGTCCT GGTGCAGGTT TTGCAGGAAT  
1601 TCAGCTCAGC AGAGTAACAA GAAACAACGC AGGGGACACA AACAAATGAGA  
1651 TTACACCTGA TCATAACAAC TGAGCAGAGA TATTATCTTT TCCATTTAGA  
1701 GGATCATCAT CAGATTTTAG CTTCAAGGTC CGGTTTTGTG GTTTATACAT  
1751 AAGTTATAGT GACTTGATTT TTTTGTTTTG TTACAAAGTT ACCATCTTTG  
1801 GATTAGAATT GGGAAATTGA ATCTGTTTGT ATATTGTATT ATTTGGAACA  
1851 TTGTGGATGC CCATGGATAT GTTCTGTTC

1 MAGGRSGSRE LPETPTWAVA VVCAVLVLVS AAMEHGLHNL SHKTTAEVLI  
51 FLVLSALAE LMLGFISLLL TVAQAPISKI CIPKSAANIL LPCKAGQDAI  
101 EEEAASGRRS LAGAGGGDYC SKFDGKVALM SAKSMHQLHI FIFVLAVFHV  
151 TYCIITMGLG RLKMKKWKKW ESQTNSLEYQ FAIDPSRFRF THQTSFVKRH  
201 LGSFSSTPGL RWIVAFFRQF FGSVTKVDYL TMRQGFINAH LSQNSKFDFH  
251 KYIKRSLEDD FKVVVGISLP LWFVGILVLF LDIHGLGTLI WISFVPLIIV  
301 LLVGTKLEMV IMEMAQEIQD RATVIQGAPM VEPSNKYFWF NRPDWVLFFI  
351 HLTLFHNAFQ MAHFVWTMAT PGLKKCFHEN IWLSIVEVIV GISLQVLC SY  
401 ITFPLYALVT QMGSNMCKTI FEEQTMKALM NWRKKAMEKK KVRDADAFLA  
451 QMSVDFATPA SSRSASPVHL LQVTGRVGRP PSPITVASPP APEEDMYPVP  
501 AAAASRQLLD DPPDRRWMA SADIADSDF SFSAGR\*

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1 MAGPAGGREL SDTPTWAVAV VCAVMILVSV AMEHALHKLK HWFHKWRKKA  
51 LGEALEKMKA ELMLVGFISL LLIVTQDPVS RICISKEAGE KMLPCKPYDG  
101 AGGGKGKDNH RRLLWLQGES ETHRRFLAAP AGVDVCAKQG KVALMSAGSM  
151 HQLHIFIFVL AVFHVLYSVV TMTLSRLKMK QWKKWESETA SLEYQFANDP  
201 SRCRFTHQTT LVRRHLGLSS TPGVRWVVAF FRQFFTSVTK VDYLTLRQGF  
251 INAHLSQGNR FDFHKYIKRS LEDDFKVVVR ISLKLWVAV LILFLDFDGI  
301 GTLLWMSVVP LVILLWVGTK LEMVIMEMAQ EIHDRSVVK GAPAVEPSNK  
351 YFWFNRPDWV LFLMHLTLFQ NAFQMAHFVW TVATPGLKKC YHEKMAMSIA  
401 KVVLGVA AQI LCSYITFPLY ALVTQMGS HM KRSIFDEQTA KALTNWRKMA  
451 KEKKKARDAA MLMAQMGGGA TPSVGSSPVH LLHKAGARSD DPQSVPASPR  
501 AEKEGGGVQH PARKVPPCDG WRSASSPALD AHIPGADFGF STQR\*

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**Figure 15**

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1  MCHGGESEMSL EFTPTWVWAG VCTVIVAISL AVERLLHYFG TVLKKKKOKP
51  LYEALQKVKE ELMLLGFISL LLTVFQGLIS KFCVKENVLM HMLPCSLDSR
101 REAGASEHKN VTAKHEFTQT LPIVGTTRRL LAEHAAVQVG YCSEKEKVPL
151 LSLEALHHLH IFIFVLAISH VTFCVLTVIF GSTRIHQWKK WEDSIADKFK
201 DPETALRKRR VTIVHNHAFI KEHFLGIGKD SVILGWTQSF LKQFYDSVTK
251 SDYVTLRLGF IMTHCKGNPK LNFHKYMMRA LEDDFKQVVG ISWYLVIFVV
301 IFLLLVNNGW HTYFWIAFIP FALLLVAVGK LEHVIAQLAH EVAEKHVAIF
351 GDLVVKPSDE HFWFSKPQIV LYLIHFILFQ NAFELAPFFW IWVTYGFDSK
401 IMGQVRYIVP RLVICVFIQV LCSYSTLPLY AIVSQMGSSF KKAILEENVQ
451 VGLVGWAQKV KQKRDLCAAA SNGDEGSSQA GPGPDGSGS APAAGPGAGF
501 AGIQLSRVTR NNAGDTNNEI TPDHNN*
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**FIGURE 16**

[illegible]

FIGURE 16 (CONT/D)

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Hvml0-H1	AQILCSYITF	PLYALVTQMG	SHMKRSIFDE	QTAKALTNNWR	KMAKEKKKAR	DAAMLMAQMG	
Mlo	LQFLCSYMTF	PLYALVTQMG	SNMKRSIFDE	QTSKALTNNWR	NTAKEKKKVR	DTMLMAQMI	
Osmlo-H1m	LQVLCSYITF	PLYALVTQMG	SNMKKTIFEE	QTMKALMNNWR	KKAMEKKKVR	DAFLAQMIS	
Atmlo-H1	IQVLCSYSTF	PLYALVTSQMG	SSFKKKAIFFEE	NVQVGLVGMWA	QKVQKQKRD LK	AAASNGDEGS	
Consensus	-Q-LCSY-TF	PLYALVTQMG	S-MK--IF-E	QT-KAL-NWR	--AKEKKK-R	DA--AOM-	
Hvml0-H1	GGAT	PSVGSSPV	HLLHKAGARS	DDPQSVPA SP	RAEKEG	GGVQHPARK	
Mlo	GDAT	PMPSSRGSSPV	HLLHKGMGRS	DDPQSSAPTSP	RTQQEARDMY	PVVVAHPVHR	
Osmlo-H1m	VD	PASSRSASPV	HLLQ.VTG RV	GRPPSPITVA	SPAP EEDMY	PVPA AASRQ	
Atmlo-H1	SQA GPGPDSG	SGSAPAAIGP	...GAGFA	GIQLSRVTRN	NAGDTNNEIT	PDHNN*	
Consensus	-A-----	--S--SPV	HLL-----GR-	--P-S--T-	--	P-----	
Hvml0-H1	V..P	SASSPALDAH	IPGADFGFST	DDPQR*			
Mlo	L..NP	SASSSALEAD	IPSADFSFSQ	DDPG*			
Osmlo-H1m	LLDDPPDRRW	MASSS...AD	IADSDFSFSA	DDPQR*			
Atmlo-H1	...	...	...	...			
Consensus	---P-D---	-ASS-----A-	I---DE-FS-	---			

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification</b> <sup>6</sup> : <b>C07K 14/415, C12N15/29, 15/82 //</b> <b>A01H 5/10</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 98/04586</b> <b>(43) International Publication Date:</b> 5 February 1998 (05.02.98)									
<b>(21) International Application Number:</b> PCT/GB97/02046 <b>(22) International Filing Date:</b> 29 July 1997 (29.07.97)  <b>(30) Priority Data:</b> <table border="0" style="width: 100%;"><tr><td style="width: 30%;">9615879.5</td><td style="width: 40%;">29 July 1996 (29.07.96)</td><td style="width: 30%;">GB</td></tr><tr><td>9622626.1</td><td>30 October 1996 (30.10.96)</td><td>GB</td></tr><tr><td>9704789.8</td><td>7 March 1997 (07.03.97)</td><td>GB</td></tr></table> <b>(71) Applicant (for all designated States except US):</b> JOHN INNES CENTRE INNOVATIONS LIMITED [GB/GB]; Norwich Research Park, Colney Lane, Norwich NR4 7UH (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SCHULZE-LEFERT, Paul, Maria, Josef [DE/GB]; Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH (GB). PANSTRUGA, Ralph [DE/GB]; Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH (GB). BÜSCHGES, Rainer [DE/GB]; Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH (GB).  <b>(74) Agents:</b> WALTON, Seán, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).		9615879.5	29 July 1996 (29.07.96)	GB	9622626.1	30 October 1996 (30.10.96)	GB	9704789.8	7 March 1997 (07.03.97)	GB	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 5 March 1998 (05.03.98)
9615879.5	29 July 1996 (29.07.96)	GB									
9622626.1	30 October 1996 (30.10.96)	GB									
9704789.8	7 March 1997 (07.03.97)	GB									
<b>(54) Title:</b> POLYNUCLEOTIDE AND ITS USE FOR MODULATING A DEFENCE RESPONSE IN PLANTS  <b>(57) Abstract</b>  The cloning of the <i>Mlo</i> gene of Barley, <i>mlo</i> mutants, and homologues from other species including rice and <i>Arabidopsis thaliana</i> enables manipulation of pathogen defence responses in plants. Nucleic acid and polypeptides are used in the production of transgenic plants in which the pathogen defence response is modulated, particularly stimulated. Various approaches enable diagnostic determination of the presence of susceptibility or resistance alleles in plants. Compounds able to modulate a defence response by interaction with Mlo or mlo protein are identifiable using assays involving the protein or fragments thereof.											

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# INTERNATIONAL SEARCH REPORT

Application No  
PCT/GB 97/02046

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/415 C12N15/29 C12N15/82 //A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HINZE K ET AL: "RESTRICTION FRAGMENT LENGTH POLYMORPHISM-MEDIATED TARGETING OF THE ML-O RESISTANCE LOCUS IN BARLEY (HORDEUM VULGARE)"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,</p> <p>vol. 88, May 1991, WASHINGTON US,</p> <p>pages 3691-3695, XP002035298</p> <p>cited in the application</p> <p>see abstract</p> <p style="text-align: center;">--- -/--</p>	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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Date of the actual completion of the international search

16 December 1997

Date of mailing of the international search report

20.01.98

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## INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCT/GB 97/02046

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TANKSLEY S D ET AL: "CHROMOSOME LANDING: A PARADIGM FOR MAP-BASED GENE CLONING IN PLANTS WITH LARGE GENOMES" TRENDS IN GENETICS., vol. 11, no. 2, February 1995, AMSTERDAM NL, pages 63-68, XP002006911 cited in the application see the whole document	1
A	--- BECKER J ET AL.: "COMBINED MAPPING OF AFLP AND RFLP MARKERS IN BARLEY" MOLECULAR AND GENERAL GENETICS., vol. 249, no. 1, 1995, BERLIN DE, pages 65-73, XP002049470 cited in the application see the whole document	1
A	--- WO 95 31564 A (THE GATSBY CHARITABLE FOUNDATION) 23 November 1995 see page 4, line 23 - page 6, line 5 see page 11, line 5 - page 15, line 20	4,42-50
A	--- WOLTER M ET AL: "THE MLO RESISTANCE ALLELES TO POWDERY MILDEW INFECTION IN BARLEY TRIGGER A DEVELOPMENTALLY CONTROLLED DEFENCE MIMIC PHENOTYPE" MOLECULAR AND GENERAL GENETICS., vol. 239, 1993, BERLIN DE, pages 122-128, XP002035300 cited in the application see abstract	1
A	--- JOERGENSEN J H: "DISCOVERY, CHARACTERIZATION AND EXPLOITATION OF MLO POWDERY MILDEW RESISTANCE IN BARLEY" EUPHYTICA, vol. 63, 1992, DORDRECHT NL, pages 141-152, XP002035755 cited in the application see the whole document	1
A	--- EMBL SEQUENCE DATABASE, HEIDELBERG, DE 27 JUNI 1994, ACCESSION NO. T22145, ARABIDOPSIS THALIANA CDNA CLONE 97N8T7 XP002049473 see abstract	13
P,X	--- BUESCHGES R ET AL: "THE BARLEY MLO GENE: A NOVEL CONTROL ELEMENT OF PLANT PATHOGEN RESISTANCE" CELL, vol. 88, no. 5, 7 March 1997, NA US, pages 695-705, XP002035301 see the whole document	1-68
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# INTERNATIONAL SEARCH REPORT

Application No  
PCT/GB 97/02046

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>SIMONS G ET AL.: "AFLP-BASED FINE MAPPING OF THE MLO GENE TO A 30-KB DNA SEGMENT OF THE BARLEY GENOME" GENOMICS, vol. 44, no. 1, January 1997, SAN DIEGO US, pages 64-70, XP002049472 see the whole document</p> <p>-----</p>	1-14

# INTERNATIONAL SEARCH REPORT

Inte. application No.

PCT 97/02046

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 70, 71 and 73  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
  
Lack of technical disclosure (Art. 6 PCT; PCT Search Guidelines, Chapter III 3.7)
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/02046

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9531564 A	23-11-95	AU 2415495 A	05-12-95
		EP 0759086 A	26-02-97
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